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THE PHYSIOLOGY OF THE ATRIO-VENTRICULAR CONNECTION IN THE TURTLE

II. Irritability and Rhythm Formation

HENRY LAURENS

Osborn Zoölogical Laboratory, Yale University

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INTRODUCTION

After the right and left parts of the A–V funnel of Malacoclemmys geographica had been shown to more easily conduct the excitation wave than the other parts (1), the continuation of the study of this physiological differentiation involved the investigation of a possible differentiation in irritability and in rhythm forming power. It seemed reasonable to expect that if certain portions of a muscular connection can conduct better than others, they might be more easily directly stimulated. The investigation of the rhythm forming power was suggested by the observations on ventricular rhythm described in an earlier paper (2) and the appearance of funnel rhythm in the heart of Malacoclemmys (1). It was decided also to determine the influence of the cardiac nerves on this property of the funnel fibers with particular reference to possible differences between the effects of the nerves of the right and left side.

METHODS

For testing the irritability of various portions of the heart, induction shocks were sent into it through small steel needle electrodes. Three to five pairs of these were used at one time and were stuck through

the parts of the heart the irritability of which was to be tested, and so connected with switches that they could in turn be connected with a single Harvard inductorium fed by a "Franco" radio-nitrogen battery with a potential of approximately three volts. Experiments were carried out on beating (in situ and excised) and on still (sinusless) hearts. All but three of the experiments were performed on the heart of Malacoclemmys. Of these three two were made on the snapping turtle (Chelydra serpentina) and one on the painted turtle (Chrysemys picta).

THE COMPARATIVE IRRITABILITY OF THE RIGHT, LEFT AND MIDDLE PORTIONS OF THE A–V CONNECTION

Haberlandt (3) found differences between the irritability of the funnel and of the right and left parts of the base of the V in the still frog heart. His method of experimentation with single shocks is open to criticism, because it is difficult to see from the manner in which he connected the four electrodes used for stimulating, and from our knowledge of the union of the A–V funnel with the ventricle and its relation to the bulbus musculature, how he could avoid stimulating the funnel under any conditions. Nevertheless, he found that the threshold value of the funnel was lower than that of the base of the ventricle, the difference being particularly large on the right which he explains by the assumption of an escape of current to the funnel. From Nakano's results (4) on differences in conductivity of the various portions of the funnel—the right, left and dorsal portions showing this function more perfectly developed than the others—we might have expected differences also in irritability. Perhaps Haberlandt's results are to be interpreted as indicating a higher irritability of the middle portion of the funnel than that of the right and left.

In a preliminary series of experiments the irritability of various parts of the still heart was compared. Some of the results are summarized in table 1. Briefly stated they show the funnel to have a lower threshold, (as indicated by the strength of stimulus necessary to produce a contraction) for both single (quick make and break) and interrupted stimulation, than the right and left parts of the base of the V. If the funnel is stimulated lower down it is found that the stimulus necessary to produce a contraction of the V is greater. On the other hand the irritability of the A–V connection just above the A–V boundary is higher than of the portion just below.

After the funnel fibers had been thus found to be more irritable than those of the V it was possible to proceed to the investigation of the comparative irritability of the different parts of the funnel. In most of the experiments three pairs of electrodes were used. They were placed in pairs in two parallel rows across the heart just below the A–V boundary and as nearly equally spaced as possible, so that the pair on the right and left were in the right and left side of the funnel while the middle pair was half way between them. The results of these experiments are to be seen in table 2. In nearly every case

TABLE 1

The irritability of the A–V connection compared with that of the base of the V

	SINGLE					FARADIC				
No.	Left base of V	Right base of V	Funnel just above A-V boundary	Funnel just below A-V boundary	Funnel lower down	Left base of V	Right base of V	Funnel just above A-V boundary	Funnel just below A-V boundary	Funnel lower down
23	6.25	6.0		7.0		6.75	6.75		8.0	
24	6.5		7.75	7.50		7.0		9.0	7.75	
25	6.0	6.5		7.5	5.75	7.0	7.0		8.0	6.5
26	7.5	7.5	8.75	8.0	7.25	8.5	8.0	9.5	9.0	8.0
27	7.0	7.0		8.5	7.75	7.5	7.5		9.0	8.5
28	6.5	6.25	10.0	7.75		7.25	6.75	10.75	8.5	
29	6.0	6.5	8.0	7.5	6.0	6.5	7.25	8.5	8.25	7.25
30	5.0	5.75		8.0		6.5	7.5		8.75	
31	6.5	6.0	8.5	8.0	7.75	7.0	6.5	9.25	8.75	8.25
66	7.0		9.5	9.0		8.5		11.0	10.25	
67	6.5		7.5	6.5		7.5		8.0	6.75	
68	8.5	8.0	11.0	10.0		8.75	8.5	12.0	11.25	
69			9.5	8.0				11.25	9.5	
70			10.0	10.0	8.0			11.0	11.0	8.5
71			8.5	8.0	7.0			10.25	8.75	7.5
72			9.5	8.5	8.0			10.75	9.0	8.5

the strength of stimulus necessary to produce a contraction of the still A and V, or standstill or an extra systole in the beating heart, is greater when sent into the funnel by the middle pair of electrodes than when sent in by either of the other two pairs. The differences are more evident in the still than in the beating heart. In some cases the irritability indicated by the right and left pairs of electrodes was lower than that indicated by the middle pair. It was found that by moving the outer pairs of electrodes nearer in, a higher threshold value was usually to be obtained. This led to the carrying out of a series

of experiments on large still hearts in which five pairs of electrodes were used. These were stuck in pairs through the right, the right middle, the middle, the left middle and the left of the base of the V just below the A–V boundary. The results of these experiments may conveniently be placed in two groups. In the first are those in which the irritability of the portions stimulated by the two outermost pairs of electrodes is highest with a gradual decrease toward the middle (table

TABLE 2

Comparative irritability of the left, middle and right part of the A–V connection

NO.	BEATING						STILL					
	Single			Faradic			Single			Faradic		
	Left	Middle	Right	Left	Middle	Right	Left	Middle	Right	Left	Middle	Right
32	5.5	6.0	7.0	6.0	6.5	7.0	7.25	6.0	8.75	7.5	7.5	8.75
33	5.75	5.0	7.0	6.5	5.5	7.5	7.0	6.25	8.0			
34	6.0	6.5	6.5	7.0	7.5	7.0						
35	8.25	7.0	7.5	8.25	7.25	8.0				9.0	7.5	8.5
36	7.5	6.0	7.75	8.5	6.75	7.5	8.5	6.75	7.5			
37	7.0	6.5	7.0	7.5	6.5	6.5						
38	6.0	5.5	6.25	8.0	6.5	8.5						
39	5.0	5.0	5.0	5.5	4.75	5.25	7.0	6.75	6.5	7.5	6.5	6.75
40	5.75	5.5	6.5	6.75	5.5	6.0	7.5	5.5	7.0	8.5	7.25	8.25
41	8.5	7.5	8.25	9.25	8.25	9.0						
42	6.0	6.0	6.5	6.5	6.0	7.0						
43	6.5	6.0	6.0	7.0	6.75	6.75	8.25	6.75	7.75	8.75	7.25	8.25
44	6.75	6.0	6.0	7.5	7.0	6.75						
45	6.0	5.0	7.0	5.5	5.5	6.75	6.0	4.5	6.0	8.75	6.5	9.75
46							6.5	5.5	7.0	7.5	6.5	6.75
47							4.0	3.0	6.5	6.0	4.5	7.0
49	5.75	5.5	5.5	7.0	5.0	6.0	8.75	6.0	7.5	10.5	7.5	8.75
Snapper 1	7.25	7.5	8.0	8.75	8.0	9.25						
Snapper 2	7.0	6.5	8.5	8.5	6.75	10.0						
Painted 1	6.5	5.25	6.25	6.75	6.0	7.0	8.0	6.0	8.5	11.0	7.5	10.75

3). In the second are those in which the irritability indicated by the outermost pairs is lowest and in which the irritability indicated by the right middle and left middle electrodes is the highest (table 4). These results lead to the assumption that in the cases given in table 3 the outside pairs of electrodes were really stuck through the right and left edges of the funnel, and that in the cases listed in table 4 these electrodes were in the base of the V outside the funnel.

TABLE 3

The comparative irritability of the A–V connection

NO.	SINGLE					FARADIC				
	Left	Left middle	Middle	Right middle	Right	Left	Left middle	Middle	Right middle	Right
56	9.25	7.5	6.75	7.5	8.75	10.75	8.25	7.5	8.25	9.5
61	7.75	6.75	6.5	6.75	7.5	9.5	7.75	7.75	8.0	8.25
62	8.0	7.5	6.75	7.75	8.5	9.5	8.5	7.5	8.5	10.0
66	7.5	7.25	6.5	7.5	7.5	9.0	7.75	7.5	7.75	9.5
69	8.5	7.5	6.75	7.0	7.75	10.0	8.25	7.75	8.0	9.75

TABLE 4

The comparative irritability of the A–V connection and of the left and right base of the V

NO.	SINGLE					FARADIC				
	Left	Left middle	Middle	Right middle	Right	Left	Left middle	Middle	Right middle	Right
58	6.0	8.25	7.25	8.75	5.75	6.75	9.5	7.5	9.25	7.0
65	5.25	8.0	6.75	7.5	5.5	6.0	10.25	7.5	10.75	6.25
67	6.5	7.5	7.0	7.25	6.5	7.5	8.5	7.75	9.0	7.25
68	6.5	7.5	8.0	7.5	6.0	7.75	9.25	8.25	8.75	7.5
75	6.25	8.75	7.0	7.5	6.5	7.0	10.5	7.75	10.75	7.0

RHYTHMIC IMPULSE FORMATION IN THE A–V CONNECTION

That rhythmic impulses due to extraneous stimulation can arise in the A–V connection of the cold blooded heart has been known for some time. Gaskell (5) observed that mechanical stimulation of the A–V ring of the still frog's heart set up a series of rapid rhythmical contractions, while stimulation of the A or of the V produced but a single contraction. Ewald (6) also found that stimulation of the funnel by piercing it with a needle resulted in a long series of contractions. Lohmann (7) pointed out that faradic stimulation of the apex of the turtle heart set up automatic contractions of the V, which were soon displaced by the Si rhythm. Also (8) that during vagal standstill one or two contractions, the impulses for which arose in the A–V connection, might appear. Gewin (9) observed, following electrical stimulation of the V of the turtle, simultaneous contractions of the V and A. And most recently Haberlandt (3) has made a study of the effects of electrical stimulation of the funnel of the frog. He found

that when the needle electrodes were stuck through the funnel, there resulted a larger or smaller number of contractions which might develop into a long series. From two to twenty contractions might result from a single electric shock. Furthermore, that it was occasionally possible by a double shock to reverse for a considerable time the sequence of the beating heart, and that this might also be accomplished by the mere sticking in of the electrodes. Faradic stimulation was more effective than single shocks in the production of numerous automatic pulsations which assumed different forms and lasted for varying lengths of time. In a later paper (10) he found that faradic stimulation of the funnel of the turtle also might result in a funnel rhythm of, on the average, very short duration. Haberlandt (3) thinks that the effects of temperature in producing a rhythm of the still frog's heart are to be referred to effects on the funnel. Local warming of that alone had results, the local warming of the region just under the bulbus and of the apex being without results. By cooling the Si and at the same time warming the funnel he was able, in a few cases, to initiate a funnel rhythm.

EXPERIMENTAL

1. Experiments on beating hearts

a. The effects of single shocks.—Three pairs of electrodes arranged as above described were used to find out whether some part of the connection was more efficient than others in the formation of automatic rhythmic impulses. A few experiments on stimulating the still heart were made in 1912 in connection with my work on the lizard and turtle heart (2), but no conclusive results were then obtained.

Only one case of the production of a funnel rhythm following stimulation with a single induction shock has been obtained. This was in an excised heart, the funnel of which had been previously stimulated many times and by strong interrupted currents as well as single shocks. This had produced hindrances to conduction and a pulsus alternans had developed (see fig. 1). The V pulsated rapidly for about ten seconds, and then beat more slowly but irregularly, the impulses, for some of the contractions at any rate, coming from the funnel. It is impossible to say positively that the A contractions originated in the funnel, but they probably did, judging from their decreased frequency and the pause just before the Si rhythm returned (see *).

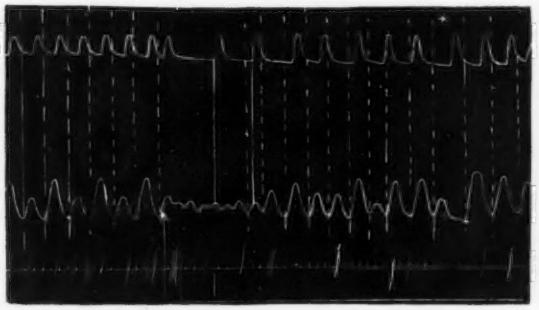

Fig. 1. "Funnel rhythm" resulting from a double induction shock to the funnel.

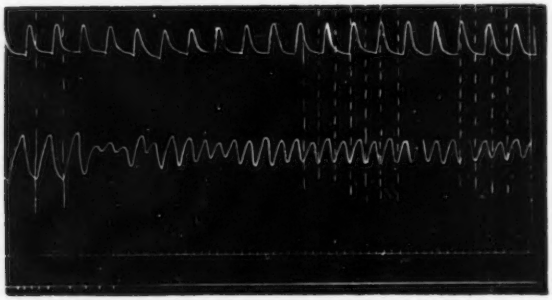

Fig. 2. Rapid V rhythm resulting from four double induction shocks to the funnel.

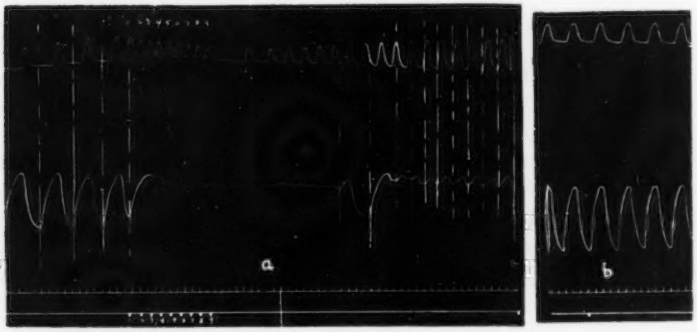

Fig. 3, *a*, V fibrillation and rapid A rhythm followed by rapid V-A rhythm resulting from nine double induction shocks to the funnel; *b*, normal Si rhythm.

Quite often when the electrodes are stuck into the funnel just below the A–V boundary there is a cessation of the rhythm for a short while, or a V fibrillation is caused which may last for a few seconds, or a rapid rhythm (flutter) which involves both V and A may be developed. But all of these are of short duration.

The effect of a series of single shocks was tried. In a few cases results of interest were obtained but only in a very few did the effects last over after the stimulation. Figure 2 shows the production in this manner of a rapid V rhythm which lasted for seven minutes, the A being unaffected and beating normally throughout. Figure 3 illustrates the production by a series of single shocks of a V fibrillation followed, after a pause, by a rapid rhythm. The A was influenced in this case, beginning to beat rapidly in response to impulses from the funnel during the V fibrillation. This rapid funnel rhythm lasted for five minutes at the end of which time it was stopped by jarring the table, being followed after a pause by the normal Si rhythm shown in the second part of the figure.

Haberlandt, as above mentioned, found that he could reverse by a single induction shock the sequence of beat of the frog's heart. He figures such a case in his figure 2. His argument (p. 17) against this being a case of retarded normal conduction is not convincing. In the first place the agreement in the time relations of the rhythm before and after the stimulation is against it as well as the appearance of block. Instead of, as he considers, having a case of a gradually wandering upward of the seat of origin of these impulses in the funnel it seems to me that we have here a case of a gradually increasing length of conduction time until block, as shown in his figure 3, appears. As he himself admits, the retarded conduction and the block are due to mechanical injury caused by the electrodes and the electric stimulation, although he found that conditions of block could be improved, and the still V be made to beat again and following the A, by stimulation of the funnel. Haberlandt found also that stimulation with a single make and break shock could produce, sometimes after a long latent period, the formation in the funnel of series of long lasting automatic impulses which began usually with incomplete and complete tetanus of the V and fibrillation, and later characterized by very frequent and regular contractions of the V but showing no influence on the A. It should be noted in referring to Haberlandt's figures 6, 7 and 8, which he gives as illustrative of these conditions, that A–V block existed or had existed. He obtained one case of this sort of

thing with a sinusless heart and admits that the block in the beating heart may have been of assistance in producing these results, though not absolutely necessarily so, since he claims to have produced the same thing in a normally beating heart (see his figure 7). The heart on which this result was obtained had, however, earlier shown A–V block. Haberlandt also argues against the dependence of the formation of these effects on intracardial vagal stimulation. A glance at his figure 8 will show that there was also a negative inotropic effect on the A in this case.

b. The effects of interrupted currents. It has been found that faradic currents sent into the funnel of excised hearts may occasionally start rhythmic impulse formation there which expresses itself in one of several different forms of contraction, which, within limits, may change from one to another. Usually, however, the influence of the stimulation stops with it, the Si rhythm, after a pause, reasserting itself. Perhaps the comparatively numerous results which Haberlandt obtained on the frog's heart find their explanation in the fact that the hearts were old and the strength of the Si impulses therefore weakened. The conditions of block which he describes make this seem probable, or that the impulses could not get to the V owing to lowered conductivity in the connection. In his experiments on the turtle heart (10) he describes the results of experiments carried out on a turtle in its third day of experimentation. Be this as it may it has so far proven impossible to initiate even by the strongest interrupted currents the formation of automatic impulses in the funnel of the intact heart of Malacoclemmys. The fibrillation of the V alone, or of the A and the V, stops with the stimulation and after a longer or shorter pause the normal Si rhythm returns. Such experiments were carried out on relatively fresh hearts, never later than four hours after removal of the plastron.

The results that have been obtained with excised hearts are summarized in table 5. It should be noted that when impulses can be started in the funnel in a heart they can be started again and again, not only by stimulating the funnel, but the A and the V as well.

Usually the stimulus must be of great strength and of long duration, although occasionally a strong short stimulation has been able to start automatic impulse formation. This has been found particularly in hearts in which by previous stimulation automatic impulses have been formed, and favors the view that once the impulse formation is initiated it is easier to do so again. The results which Haberlandt reports to

TABLE 5

Summary of results obtained by stimulating the A–V connection of the beating excised heart with an interrupted current

NO.	STRENGTH OF STIMULUS	LENGTH OF STIMULUS	RESULT	DURATION	REMARKS
		seconds			
35(5)a	4.0	1	V fibrillation, followed by V rhythm after gradual slowing. A fibrillation	27 sec.	
35(5)b	2.0	0.5	V fibrillation for 5 sec. followed by V–A rhythm	33 min.	
37	3.0	9	V fibrillation, and 2 automatic V systoles	14 sec.	
37(4)	0.0	9	Rapid V rhythm of 9 contractions	18 sec.	
50(3)a	4.0	8	V standstill, followed by 2 large systoles then rapid rhythm of 6 contractions	19 sec.	Figure 6. The A contractions very weak
50(3)b	4.0	17	Rapid V rhythm, S[i] rate slightly increased	110 sec.	Figure 5
50(4)a	3.0	2	V standstill followed by 6 quick V contractions	13 sec.	Cf. figure 6
50(4)b	3.0	14	V standstill followed by 2 large V systoles then rapid V–A rhythm of 5 contractions	8 sec.	Cf. figure 6
78(2)a	1.0	11	V fibrillation for 5 sec. followed by irregular V–A rhythm (10 sec.), A then fibrillates followed by regular V–A rhythm for 17 seconds	32 sec.	
78(2)b	4.0	8	Rapid V rhythm, while A fibrillates	13 sec.	
78(3)a	4.0	9	Rapid V rhythm, A fibrillates, then still	16 sec.	
78(3)b	4.0	21	Rapid V rhythm, A fibrillation	24 sec.	Figure 4

TABLE 5—Continued

NO.	STRENGTH OF STIMULUS	LENGTH OF STIMULUS	RESULT	DURATION	REMARKS
		seconds			
78(3)c	2.0	24	Rapid V rhythm, A fibrillation, then still	18 sec.	
84(3)a	1.0	4	Very rapid V rhythm, A fibrillation, then still, followed by Si rhythm, slightly increased and irregular. V rhythm later irregular	8 min.	Figures 10 and 11
84(3)b	1.0	14	Very rapid, regular V rhythm, later irregular. A in Si rhythm, but irregular, suddenly V–A rhythm appears after a pause	15 min.	
84(3)c	1.0	14	Very rapid V rhythm, later irregular, then regular again, etc. A in normal Si rhythm	15 min.	
Snapper 2(2)	2.0	1	V fibrillation (5 min.) A quickly in rapid regular rhythm from funnel impulses. V–A rhythm	65 min.	Figures 7, 8, 9, stopped by double induction shock to funnel
2(3)	3.0	2	V fibrillation (3 min.) A more rapid, then regular V–A rhythm	54 min.	
2(5)	2.0	0.5	Rapid V rhythm (15 sec.) A fibrillation (8 sec.) then still, followed by V–A rhythm	17 min.	Stopped by double induction shock to funnel

have obtained on the frog heart with threshold, or near threshold strength of stimuli, have not been substantiated, and indeed Haberlandt makes no concise claim for this on the heart of the turtle.

The automatic impulses started by faradizing the funnel of the excised beating heart exert their influence on the A and the V in a variety of forms. They may take the form of a rapid V rhythm, started dur-

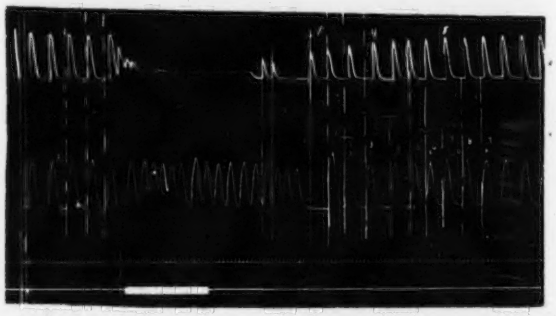

Fig. 4. Rapid V contractions and A fibrillation resulting from faradic stimulation of the funnel

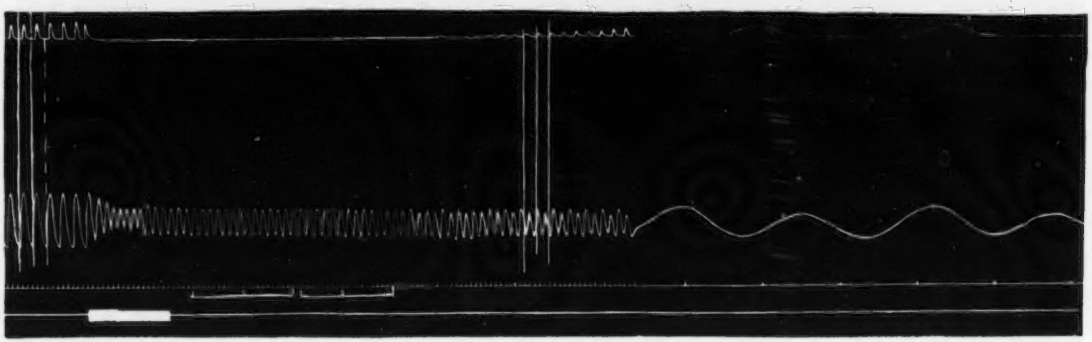

Fig. 5. Rapid V contractions, the A rate slightly increased

ing the stimulation, and consisting of separate regular pulsations which continue after the stimulation has been stopped. Figure 4 illustrates a brief case of this sort. The fibrillation of the A, as well as the negative inotropic, and perhaps also dromotropic, influence of intracardial vagal stimulation, prevents the Si impulses from reaching the funnel, thus allowing the continuation of the formation of automatic impulses there. The impulses for the two A contractions following the A fibrillation probably arose in the funnel. The formation of the funnel impulses stopped soon after this and the normal Si rhythm returned. Figure 5 shows a better example. Here the A ceases to beat (negative inotropic effect) while the V contracts rapidly, the impulses originating in the funnel continuing after the stimulation is discontinued. When the A begins to contract again, or rather when its contractions become visible again, it is seen that they are slightly more rapid than before while the V is beating almost twice as rapidly as the A. This condition lasted for a little less than two minutes, when the V paused and the Si rhythm followed.

Figure 6 illustrates how the funnel impulses for this rapid V rhythm may remain latent. The two large and the six rapid V contractions owe their origin to funnel impulses, the normal Si impulses then getting through, although they do not show on the A until later.

Faradic stimulation of the funnel may give rise to the formation there of impulses which produce a lasting over effect of V fibrillation from which various forms of rhythm may arise. The effects on the A vary. It may not be affected at all except for a slight negative inotropic effect, or its rate may be accelerated slightly. It may be brought to a standstill after a beginning fibrillation, and finally it may, after fibrillation, go over into a regular rhythmic beat which is considerably more rapid than that of the sinus, the impulses arising in the funnel. Figure 7 is a part of a typical case. This heart had been beating for some time and had previously been stimulated severely with no results. After not having been stimulated for fifteen minutes a strong interrupted current sent in for less than one second had the recorded result. Both the A and the V fibrillated, the A, however, soon beginning to beat regularly and rapidly, the impulses for the rhythm coming from the funnel. The V continued to fibrillate for about five minutes after which, after pausing momentarily, it beat as shown in the second part of figure 7, regularly, and for the most part coordinated with the A contractions, but exhibiting pulsus alternans. The pulsus alternans disappeared about five minutes later, the transi-

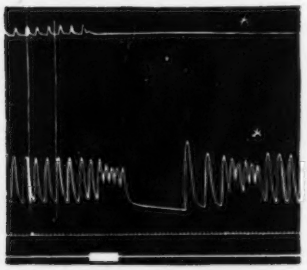

Fig. 6. Appearance of slow and rapid V contractions due to latent funnel impulses

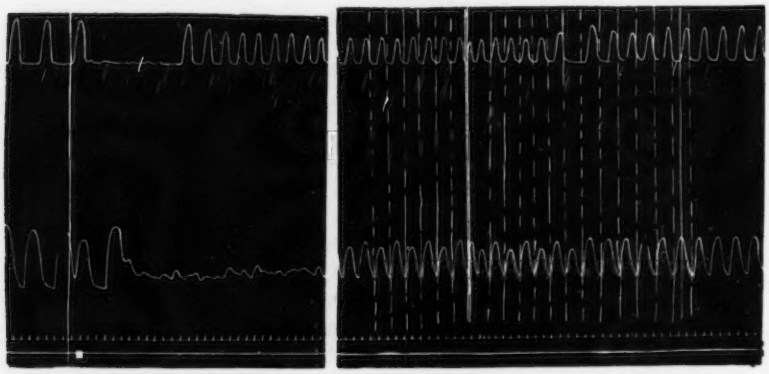

Fig. 7 *a*, V fibrillation and rapid A rhythm; *b*, V-A rhythm from funnel impulses

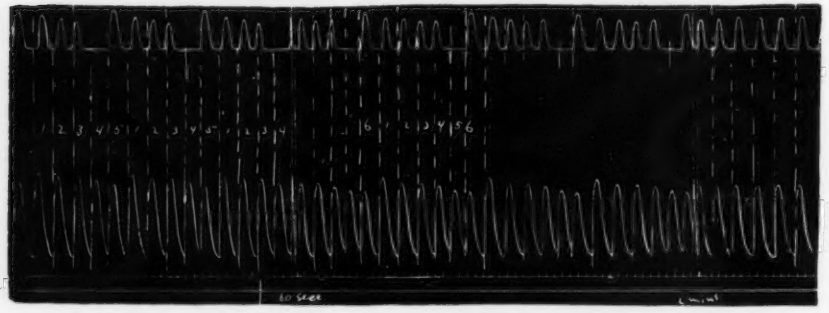

Fig. 8. Continuation of figure 7. Funnel rhythm showing V–A block

tion being shown in the figure, an As being missed after a gradual increase in vigor of contraction, following which, after a slight irregularity, the A contractions became regular and more vigorous.

Figure 8 shows a record taken of this rhythm five minutes later again. After beating regularly as shown in the last part of figure 7, a V–A block developed and the funnel impulses then arose in groups characterized by a gradual decrease in length of interval between contractions until a minimum is reached, after which there is a pause. The A–A intervals show a more rapid decrease than the V–V intervals do, and with this gradual decrease in length of interval there is a decrease in the vigor of the contractions. Beginning with systole 5, we find a maximum value between it and systole 1 for both the V and the A. This interval gradually decreases until As 4 is dropped due to refractoriness of the connection fibers, while Vs 5 follows after Vs 4 with a minimal interval. Due to the recovery in irritability, afforded by the dropping out of a beat, As 5 is most vigorous the interval being, however, greater than minimal, due, no doubt, to the weakness of the funnel impulse. After the first Vs after the pause the interval gradually decreases again and the same thing is repeated. This condition, where every fifth As is missed and where the V systoles are in groups of 5, soon improves so that every sixth As is missed and the V systoles are in groups of 6 (see middle part of figure 8). The same conditions hold here for the V as before. But when the lengths of successive A–A intervals are studied it is seen that things are a bit different. The first As after the missed beat (No. 6) still occurs in the same cycle with the Vs of minimal length of interval, while between them and the next systole (No. 1) there is the maximal length of interval which then gradually decreases with each successive cycle. The height of the V systoles also decreases regularly with the decrease in length of interval. The A systoles do so up to the last or 4th which is higher again. This fact is of interest in explaining the block and throws doubt on the assumption that the failure of the A to beat is due to the falling of the impulse into its refractory period. For if this were so it is hard to understand why the height of the last As should be more vigorous than the preceding one. There is no pause in the formation of the impulse, as the V contractions show, which would give the A fibers an opportunity to recover. It would seem that the conductivity of the funnel fibers towards the A is really decreasing from beat to beat. Through As 3 this decrease in conductivity does not become apparent owing to the gradually increasing frequency of for-

mation of rhythmic impulses in the funnel. But between As 3 and 4, owing to this decrease in conductivity in the direction of the A, there is a slight pause (A–A) during which the A fibers recover somewhat and the next As (No. 4) is therefore more vigorous. The fifth impulse in the group finds the conductivity of the connection fibers so lowered (refractoriness of conduction) that the impulse cannot get through, while the sixth impulse owing to its weakness takes longer to reach the A again. This explanation is in keeping with that offered (1) to explain the greater vigor of the last Vs in some cases of block in which there is a gradually increasing length of interval.

The greater decrease in A–A interval as compared with the decrease in V–V interval cannot be explained by assuming a gradually wandering upward of the place of origin of the impulses in the A–V connection owing to the relation between the A and V systolic interval. A record taken two minutes later than that shown in the middle part of figure 8 shows the V–A rhythm perfectly regular and slower. If one were so minded this could now be explained as a normal A–V sequence. Figure 9, a tracing taken sixty-five minutes after the stimulation, shows this not to be the case. The rate of the V–A rhythm has slowed considerably. A single make and break shock sent into the funnel blocked the funnel impulse so that it failed to reach the A, while the V contracted once again after a pause. This shock to the funnel stopped the formation of the impulses there and gave the more slowly arising Si impulses a chance to get through to the funnel, and after this the normal Si rhythm was restored.

The fourth form of contractions which the funnel impulses may give rise to are very rapid V pulsations, the A, in most cases, continuing to beat at its normal rate. These rapid pulsations of the V have been obtained in only a single heart, but in that one three times (see No. 84, table 5). Figure 10 is an example, the return to the normal Si rhythm being shown in figure 11. The rapid V contractions soon become irregular (fibrillation) though not necessarily more rapid. The A, on the whole, beats regularly and much more slowly than the V. But its rate is usually slightly increased and there are irregularities here and there which indicate some effect on the A of the impulses arising in the funnel. Sometimes the V irregularities may disappear and reappear several times before the funnel impulses give out, and one case was obtained of a V–A rhythm developing in this way (84(3)b).

Haberlandt (3) found that the various forms of contraction set up by funnel impulses in the frog could be arranged in the following order

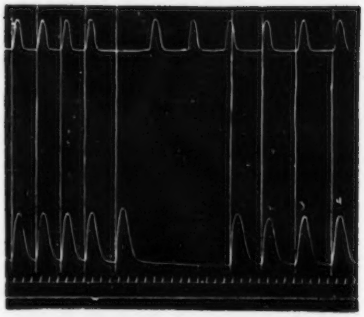

Fig. 9. Continuation of figure 8. Transition from funnel rhythm to normal Si rhythm

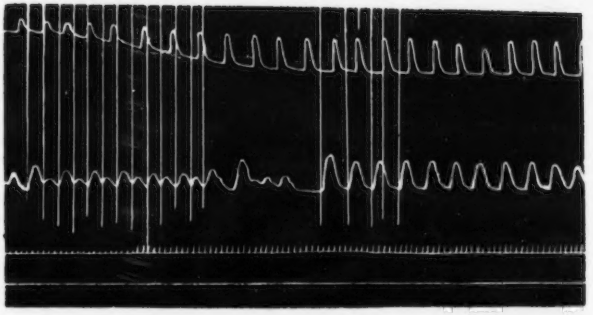

Fig. 11. Continuation of fig. 10, the giving out of the funnel impulses and the return to Si rhythm

Fig. 10. Rapid V pulsations, becoming irregular, originating in funnel impulses

according to the relative ease with which they could be obtained. (1) Very frequent independent V contractions; (2) fibrillation of the V, and (3) typical atrio-ventricular rhythm. The first two never go over to the A and the rapid V contractions sometimes develop from overlasting fibrillation.

A glance at table 5 will show, notwithstanding the relative small number of cases in which it has been possible to induce automatic funnel impulses, that the most frequent form which they give rise to is a rapid V rhythm, consisting of separate pulsations which start during the stimulation and continue after it. The A is not included in the rhythm. Next in order comes typical V–A rhythm. This is, however, not a condition which arises primarily as a result of the stimulation, but follows V fibrillation or V flutter (figs. 7, 8 and 9). Next in order of sequence is a fibrillation of the V (fig. 7) which arises from a fibrillation begun by the stimulation, or from very rapid V pulsations. And finally, the very rapid V pulsations (fig. 10) which later become irregular and which may, in slowing, give rise to a V–A rhythm.

2. Experiments on still hearts

a. The effects of single shocks. It has not proven possible to set up a rhythm in the sinusless heart by a single make and break shock sent into the funnel. Although when the needle electrodes are put in a short rhythm may be started, the frequency of beats gradually decreasing until the formation of the impulses stops. Haberlandt (3) was able by means of a single shock to start a rhythm of a larger or smaller number of contractions, their frequency being on the average the same as the normal though sometimes much less.

b. The effects of interrupted currents. It is much easier to set up a funnel rhythm in the still heart than in the beating heart. Not that it requires a weaker current, but rather that stimulation is more often attended with success than in the beating heart. In table 6 the results of the successful cases are summarized. In several cases the funnel impulse formation would have continued longer but for the fact that it was stopped by some extraneous stimulus, such as moistening with Ringer solution, or jarring the table by removing the drum, or single or interrupted shocks. Electric stimulation was undertaken in many cases to decide whether the rhythm under observation was really proceeding from impulses formed in the funnel.

As in the beating hearts the impulses formed in the funnel may cause

different kinds of results on both the A and the V. It can be stated here that the effects produced involve the A as well as the V.

In a very few cases a faradic stimulation of the funnel may result in the formation of slow rhythmic impulses which cause contractions of both the A and the V (slow V–A rhythm). Figure 12 illustrates this condition. It was obtained but twice, one of these being with a heart of the painted turtle (Chrysemys picta) where it was the only result that could be produced, being however obtainable almost at will (table 6).

A faradic stimulation may sometimes start a fairly rapid V rhythm, which as soon as the current is broken becomes a regular V–A rhythm as shown in figure 13.

The most common form of contraction to which the rhythmic funnel impulses give rise is a fibrillation of the V. This in most cases changes sooner or later into a regular, slower rhythm before the formation of the impulses stops. The A is involved, and may even begin to beat regularly during the stimulation while the V fibrillates, although in most cases it fibrillates or stands still and then, when the stimulation is stopped, begins a more or less regular rhythmic beat. The rate of the V beat is usually more rapid than that of the A and sometimes in a definite ratio to it, or the contractions of the V and A may be synchronous. Figure 14 illustrates a case of this sort. A record taken two minutes later (second part of figure) shows a regular V–A rhythm, of 2:1 ratio. Several single shocks sent into the funnel produced merely extrasystoles of the A. Slower automatic impulses may be started in the funnel, the rhythm set up later developing irregularities which may disappear so that we have again a regular V–A rhythm. (see figs. 15 and 16).

Occasionally a very rapid rhythm is started by faradisation of the funnel. This arises from fibrillation and usually slows down until it stops. Figure 17 is a case illustrating a short-lived rhythm of this sort, the V and A beating in the ratio of 2 : 1. Figure 18 shows a more interesting case in which at first the V and A beat together at a very rapid rate. Later this rhythm slows down and becomes exceptional in that the V shows a lower rate for a time than the A. The first part of figure 19 is a record taken ten minutes later showing the incoördination between the V and the A disappearing. Forty-five minutes later the two parts are beating as shown in the middle part of figure 19 coördinatedly and in the ratio of 2 : 1, the V showing pulsus alternans. A minute later the electrodes were removed (see *). Two minutes

TABLE 6

Summary of results obtained by stimulating the A–V connection of the still heart with an interrupted current

NO.	STRENGTH OF STIMULATION	LENGTH OF STIMULATION	RESULT	DURATION	REMARKS
		sec.			
28(2)	4.0	5	V fibrillation, slow A rhythm, followed by slow V–A rhythm	90 min.	Stopped by double induction shock to funnel
28(4)	4.0	8	V–A rhythm, quick. Later V is irregular	30 min.	Started by stimulating base of V on right. Stopped by jarring
29(3)	5.0	12	Slow V–A rhythm, following fibrillation	4 min.	Started by stimulating base of V on left
29(4)	5.0	16	Slow V–A rhythm, following fibrillation	4 min.	Started by stimulating base of V on right. Stopped by faradic to funnel
29(5)a	5.0	13	Fibrillation of both A and V, ending in two quick V–A cycles	15 sec.	Started by stimulating base of V on right
29(5)b	5.0	15	Fibrillation of both A and V, ending with three quick V–A cycles	9 sec.	Started by stimulating base of V on left
36(3)	0.0	3	Slow V–A rhythm, followed by rapid and irregular V contractions, A regular, finally rapid V–A	19 min.	Figures 15, 16
36(4)	0.0	15	Fibrillation of both V and A, followed by very rapid V systoles, finally developing 2:1 V–A rhythm	5 min.	Stopped by jarring table
36(5)	4.0	13	Rapid and incoördinated V and A rhythm, later developing 2:1 V–A rhythm	3 min.	Figure 14

TABLE 6—*Continued*

NO.	STRENGTH OF STIMULATION	LENGTH OF STIMULATION	RESULT	DURATION	REMARKS
		sec.			
36(6)	0.0	9	Fibrillation of both, followed by V–A rhythm	4 min.	
36(7)	0.0	9	Fibrillation of both, followed by 2: 1 V–A rhythm	5 min.	
36(8)	4.0	16	Fibrillation of both, followed by V–A rhythm	9 min.	
36(9)	2.0	11	Fibrillation of both, followed by 2: 1 V–A	40 sec.	Stopped by faradic to funnel
36(10)	3.0	10	Fibrillation of both, followed by V–A	30 sec.	Stopped by faradic to funnel
39(3)	4.0	1	Slow V–A rhythm of five pulses	60 sec.	Figure 12
49(1)	1.0	11	Fibrillation of A, very rapid contractions of V, followed by V–A	9 min.	
53(2)	0.0	15	A rhythm, V fibrillation, followed by very quick V contractions giving 2: 1 V–A	86 sec.	Figure 17
57(2)	2.0	15	A fibrillation, rapid V contractions, followed by V–A rhythm	90 sec.	Figure 13
61(1)	6.0	17	Rapid V contractions becoming irregular, but coördinated with A contractions	5 min.	Figures 21 and 22. Started by stimulating right auricle
81(2)	4.0	19	Very rapid V–A rhythm, gradually slowing and becoming irregular	200 min.+	Figures 18, 19 and 20
Painted turtle, (1)	3.0	1	Slow V–A rhythm of nine pulses	65 sec.	
1(2)	5.0	1	Slow V–A rhythm	3 min.	
1(3)	5.0	1	Slow V–A rhythm	7 min.	
1(4)	4.0	1	Slow V–A rhythm	5 min.	

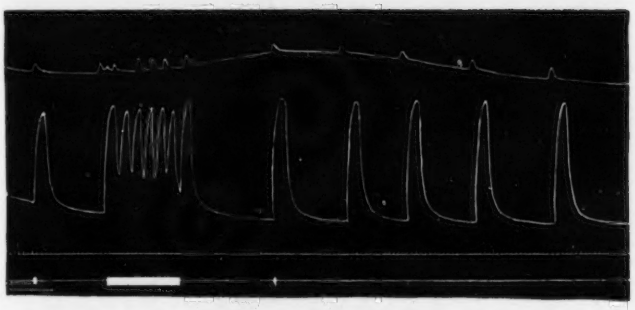

Fig. 12. Slow V–A rhythm

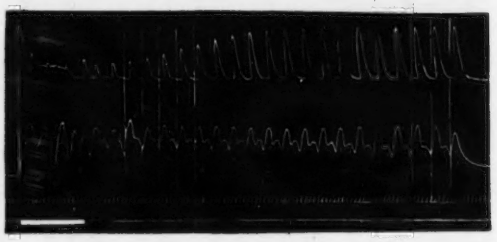

Fig. 13. V–A rhythm developing directly from rapid V contractions

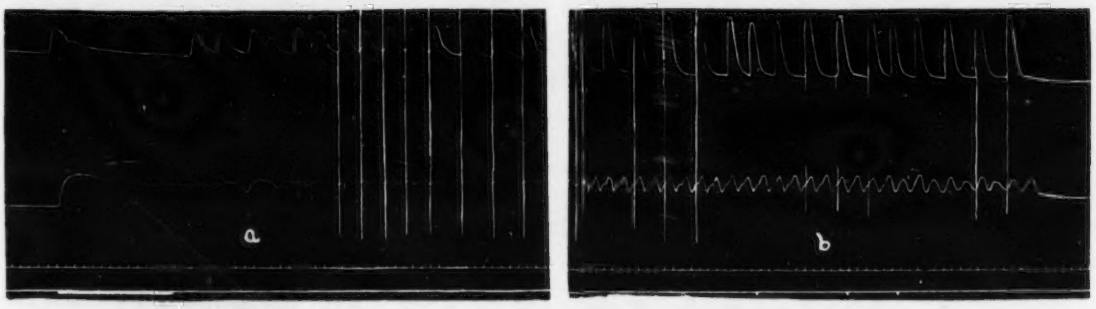

Fig. 14. The gradual development of a rapid V–A rhythm (2 : 1) after V fibrillation. Double induction shocks to funnel produce only extra systoles of the A

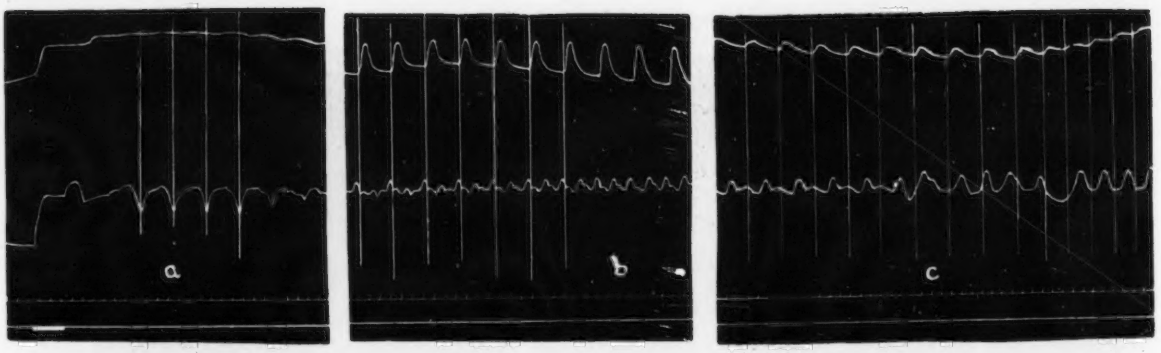

Fig. 15. Slow V–A rhythm showing V irregularities and finally becoming a regular V–A rhythm

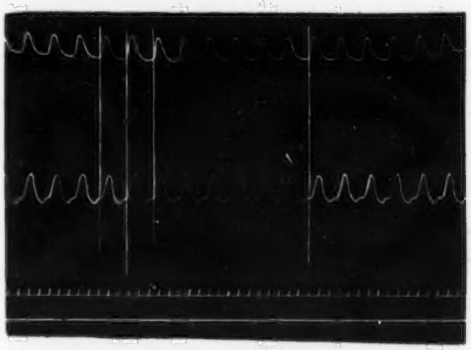

Fig. 16. Continuation of figure 15

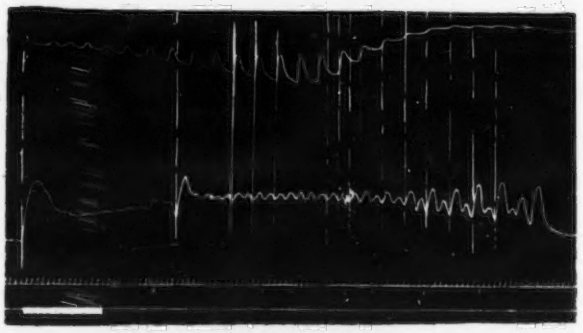

Fig. 17. Rapid V pulsations developing from V fibrillation. V–A rhythm (2 : 1)

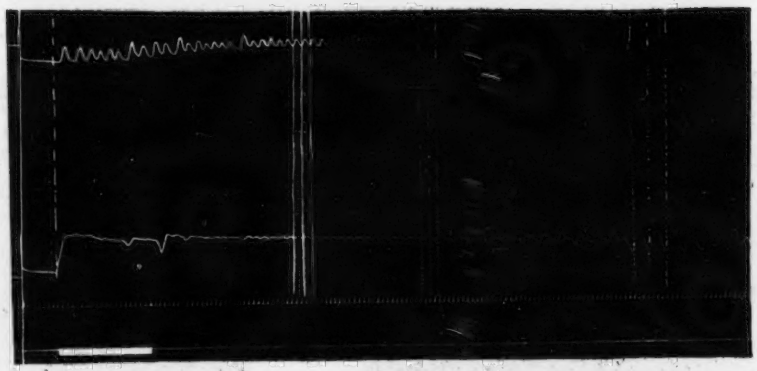

Fig. 18. Very rapid V–A rhythm developing from V fibrillation

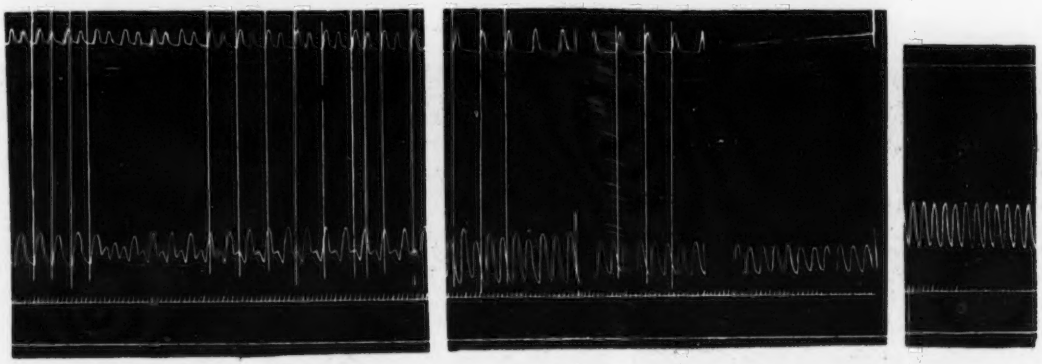

Fig. 19. Continuation of figure 18, description in text

later the A–V connection was cut through just above the base of the V (see **). The A immediately stood still while the V continued to beat. The last part of figure 19 shows a record taken seventeen minutes later (an hour and seventeen minutes after stimulation). Several attempts were made to stop this rhythm by electric stimulation (see fig. 20). At 12.52 (the stimulation having taken place at 11.15) the record shown in the second part of this figure was taken. The levers were then detached from the A and V and the two parts placed in a moist chamber. At 2.35 the V was beating between fourteen and sixteen times per minute. No further opportunity of making observations was offered until 5.25 and durng the interval the V had become still.

This "funnel rhythm" can be produced by stimulating the A or the V as well as the funnel itself. A strong current was sent into the middle portion of the funnel of a heart with the results shown in the first part of figure 21. Five minutes later the same strength of current was sent into the right auricle with the result of starting a rapid rhythm which, when the current was broken continued as a regular, rapid rhythm for about twenty-five seconds, to then become quicker and irregular, while the A began to beat more vigorously and regularly. Figure 22 is a portion of a record taken three minutes later. The V is beating irregularly and in groups, but the separate beats can be seen to be coördinated with those of the A. Soon after this the impulse formation was stopped by moistening the heart with Ringer.

In this particular case it may be a question as to where the impulse for this rhythm was being formed. Presumably in the funnel since with the strength of current used there must have been escape of current to the funnel. In several cases in which a rhythm had been started by stimulating the A in the manner just described, the effects of cutting the A–V connection were tried. Usually the rhythm stopped immediately. Occasionally, however, the V keeps on beating while the A stops. These results lead to the conclusion that, although the A was stimulated, the impulses were excited to arise not in the A but in the funnel. This matter will be referred to later.

As to the relative frequency with which the various forms of rhythmic contractions set up by automatic funnel impulses may be obtained, reference may be again made to Haberlandt for the order which he obtained in the frog. (1) The production of series of slow pulsations, (2) rapid V contractions, whose frequency is so great as not to permit of complete relaxation between them, and (3) the very frequent V contractions which may arise from overlasting fibrillation.

Fig. 20. Continuation of figure 19, description in text

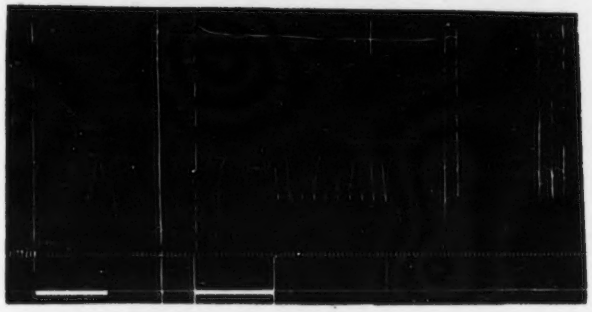

Fig. 21. Rapid V contractions arising from funnel impulses started by stimulation of the right auricle, becoming a V–A rhythm

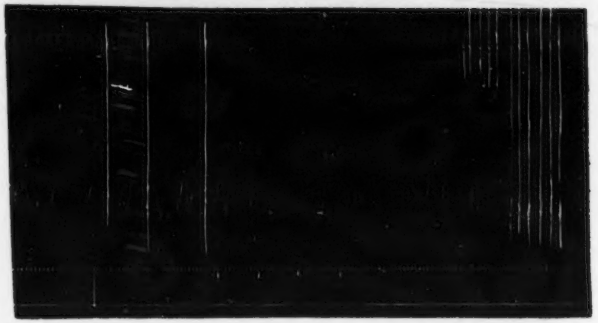

Fig. 22. Continuation of figure 21, description in text

In the still heart of Malacoclemmys the most frequent form of funnel rhythm is a fibrillation of the V which may later change into any of the others but the very slow V–A pulsations. Most frequently the fibrillation changes to a fairly rapid V–A rhythm, as in figure 14, or to a very rapid rhythm as in figures 17 and 18. Only very occasionally does the fibrillation go over into a slow V–A rhythm as in figure 15, which may arise immediately after or even during the stimulation. Sometimes the V–A rhythm develops from a rapid rhythm as in figure 13. And least frequently of all do the funnel impulses take the form of a slow V–A rhythm, as in figure 12. The A is included in all, usually coördinated with the V, and often in a 1 : 1 ratio.

DISCUSSION AND CONCLUSIONS

In addition to there being a differentiation in conductivity in the A–V connection of Malacoclemmys geographica, the same parts of the funnel, viz., the right and left, show also a differentiation in irritability, it being possible to more easily produce a contraction of the V, and of the A, by stimulating them. The differences are more clearly seen in the still heart and by using interrupted currents.

It is possible to initiate the formation of rhythmic impulses in both beating and still hearts by stimulating the funnel with interrupted currents. The impulses arise in the A–V connection and probably in the V portion of this. The rhythmic contractions which these impulses give rise to take various forms as has been described and illustrated above. It is, however, impossible to find any portion of the funnel which seems to possess this ability to give rise to automatic rhythmic impulses in greater degree than others. The strength of stimulus necessary to produce the formation of the impulses is so strong that it is impossible to contend that a certain portion only is being stimulated and that there is not an escape of current. Furthermore, stimulation of the A and of the V (apex, middle or base) can also initiate a rhythm which lasts over after the stimulation is discontinued. Haberlandt was able to show that it required stronger stimuli when the base of the V of the frog was stimulated than when the current was sent into the funnel. The rhythm set up is, however, always due to impulses arising in the funnel which is excited by the spread of the current from the place stimulated.

Single shocks are ineffective in producing a rhythm in the turtle heart except for the few cases described above, as are faradic currents

of threshold or near threshold strength. Haberlandt found that he could reverse the sequence of the beat in the frog's heart by a single shock. It is difficult to understand why it is so comparatively easy to do this when the rapidly arising impulses in the funnel resulting from faradic stimulation giving rise to the characteristic contractions of the V are not also able to suppress the Si rhythm and to control the beat of the A. Haberlandt (10) cites as corroborative evidence Eckstein's results (12) on the beating of the A in normal rhythm when the frequency of the V contractions was increased by artificial stimulation. It should be remembered, however, that Eckstein also found that the V might beat more slowly than the A when the latter was artificially stimulated, and Haberlandt also observed (10) that during overlasting fibrillation of the A, the V may beat in an apparently normal frequency, the impulses for the V contractions coming from the funnel. As above shown in the still heart of Malacoclemmys the funnel impulses always go over to the A.

Haberlandt (3) reported that sometimes by electrical stimulation of the still heart an automatic rhythm may be started which has its seat of origin in the A or in the A part of the A–V connection. He cites Engelmann's results (13) and Seemann's (14) substantiation of them with the electrocardiogram, as well as Lohmann's (15) observation, in support of his contention. Engelmann's and Seemann's results are no doubt to be taken as evidence that the "funnel rhythm" may arise in the A part of the connection, and that the impulses therefore reach the A slightly before they reach the V. This is, however, still a "funnel rhythm" and not an auricular rhythm as Seemann would have us believe it is. Lohmann's results are, it seems to me, different. By suspending the Si and A, after cutting off the V, he observed in the frog and turtle that often the registered contractions of the A were faster than, and not coördinated with those of the Si. He concludes that the A is beating in a rhythm of its own. The question as to just where the pace-maker in the turtle heart is has not been until recently, if now, definitely settled. The conflicting views on this matter are reserved for later discussion, but a possible explanation may now be put forward of the results which Lohmann obtained. In all probability Lohmann suspended a part of the Si removed from the junction with the A, possibly a part of one of the veins, in which the rate of contraction (or the rate of impulse formation) was slower and in no definite ratio to that of the pace-maker, the impulses from which were setting the pace for the A contractions. This would give rise to

the lack of coördination between the rates of contraction of the two parts which he was registering.

In addition to the experiments on stimulating the A directly, the results of which have been briefly referred to above, experiments were also made on stimulating the still A and V after they had been divided at the A–V boundary as close to the V as possible. It will be remembered that it was conclusively shown (1) that the still V placed in a moist chamber might, and usually did, develop a slow rhythm, and that the sinusless A did not. By stimulating the funnel of the isolated V by electrodes stuck through the middle of the base a rhythm could be started showing the various characteristics above described. When the A on the other hand was stimulated, whether at the A end of the funnel or elsewhere, this automatic rhythm did not result. Also in the cases mentioned above in which a rhythm had been started by the stimulation of the A, and in which the A–V connection was then cut, the A invariably stood still, while in a few the V continued to beat. All these negative results on the production of an A rhythm lead to the conclusion that the rhythm started in the heart has its origin in impulses arising in the A–V funnel and usually in that part below the level of the A–V boundary.

We have seen that it is impossible to produce this "funnel rhythm" in the intact heart by even the strongest stimuli, further that it can be occasionally produced in the excised beating heart, and most frequently in the still, sinusless heart. These facts make it seem probable that the failure of the funnel impulses to materialize is due to the presence of the Si impulses which assert themselves and blot out the funnel impulses. Furthermore in the beating excised hearts it is only in relatively old preparations that the funnel impulses seem to be able to last over for any length of time. The conclusion may therefore be drawn that the Si impulses must be weak, or that the Si impulses must be blocked from reaching the funnel in order that the funnel impulses should have a chance to get started. This may seemingly be done by causing the auricles to fibrillate or to stop beating, as the result of intracardial vagal stimulation (negative inotropic or even dromotropic influence), or as the result of a refractory condition of the A fibers caused by the rapid contractions. After the funnel impulses have gotten started they seem to have a better chance to continue, and thus set up a rhythm of the V. After this the A may then begin to beat, weakly at first, and either following the Si impulses or those of the funnel. It has again and again been observed when a rapid

regular rhythm or fibrillation of the V has been started by faradization of the funnel, that it lasts over only so long as the A contractions (from the Si) remain suppressed. As soon as the Si impulses are able to get through to the funnel the formation of the funnel impulses is stopped.

SUMMARY

1. There is a differentiation in irritability as well as in conductivity in the A–V connection of the turtle Malacoclemmys geographica, the right and left parts having a lower threshold, as indicated by the strength of stimulus necessary to produce an impulse for contraction.

2. No part of the connection has been found to possess in higher degree than others the function of impulse formation.

3. By faradic stimulation of the connection it is possible to initiate the formation of automatic impulses there which give rise to a variety of forms of contraction. Single shocks do not have this capacity, with the exception illustrated in figure 1. A series of quick double induction shocks may, however, start the formation of impulses which produce a rapid rhythmic V beat (fig. 2) or a V fibrillation and rapid A beat followed by a V–A rhythm (fig. 3).

4. The rhythmic contractions proceeding from funnel impulses in the beating heart may take the form of:

a. Rapid V contractions in which the A does not take part (figs. 4, 5 and 6), though its frequency of beat may be slightly increased.

b. V fibrillation for a longer or shorter time, which later may develop into some other form of "funnel rhythm." The A usually fibrillates also, but not for so long a time as the V, and either stands still or begins to beat rapidly and rhythmically (fig. 7a).

c. V flutter, or very rapid but regular V contractions, the A usually continuing to beat at the normal rate though slightly irregular (fig. 10) and sometimes slightly accelerated. Occasionally the A is included in the funnel rhythm. The rapid V contractions may become very irregular and remain so up to the time of disappearance, or they may again become regular and thus alternate several times.

d. Typical V–A rhythm developing secondarily from either V fibrillation (fig. 7a), or from very rapid V contractions.

5. The rhythmic contractions proceeding from funnel impulses in the still heart may take the form of:

a. Slow V–A rhythm (fig. 12).

b. V fibrillation, while the A fibrillates, or stands still or beats in an irregular rhythm. This type of funnel rhythm may give rise to any of the others, except the slow V–A rhythm (figs. 14, 15, 17, 18).

c. Typical V–A rhythm which may develop immediately after the stimulation (fig. 13) or after fibrillation and rapid V contractions (figs. 14, 15).

d. Very rapid V–A rhythm arising almost immediately after stimulation and following a very short fibrillation (figs. 17, 18).

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BILE PIGMENT METABOLISM

VI. Bile Pigment Output Influenced by the Eck Fistula

G. H. WHIPPLE and C. W. HOOPER

From The George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

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The Eck fistula presents an easy method by which the blood supply of the liver can be diminished, and changed from a mixed venous-arterial blood supply to a pure arterial circulation. The term Eck fistula in this paper means a free anastomosis between portal vein and vena cava together with ligation of the vena porta between the anastomosis and hilum of the liver. This operation deviates all the portal blood away from the liver into the vena cava and limits the blood supply of the liver to the hepatic artery. Dogs with an Eck fistula on a suitable diet may live a normal life for two years or longer.

It is not surprising that the Eck fistula liver is found to be smaller than normal—a decrease from the normal liver weight of 20 per cent on the average. When we recall that normally three-fourths of its blood is brought to the liver by the portal vein (Burton-Opitz (1)), the wonder is that the liver stands the operation as well as it does without showing more atrophy and degeneration. The Eck fistula liver of some months duration will show invariably a definite atrophy of the liver cells in every lobule. There is present, too, a definite fatty degeneration of the liver cells in the center of the liver lobules and some increase in the finely granular lipochrome pigment of the liver cells.

There is positive evidence that this atrophic Eck fistula liver is functionally inefficient. Phenoltetrachlorphthalein (2) injected into the blood is excreted in the bile, and under uniform conditions gives some evidence of the functional capacity of the liver. Liver poisons will cause a decreased output of this tetrachlorphthalein in proportion to the amount of liver injury. The Eck fistula liver shows a diminished capacity to excrete phenoltetrachlorphthalein—usually two-thirds or three-fourths normal capacity, after the liver atrophy and degeneration have had time to become established. We have anatomical as

well as functional evidence that the Eck fistula liver is functionally below normal.

We have pointed out (3) that Eck fistula dogs with obstructed common bile ducts develop a distinct icterus, which, however, is much less intense than that observed with bile duct obstruction in a normal dog. This suggested to us that the production of bile pigments might depend in part on the functional activity of the liver parenchyma. In other words, that bile pigment formation might be in part a true synthesis due to liver cell activity rather than a passive metamorphosis of hemoglobin pigment to bile pigment depending solely on the supply of hemoglobin brought to the liver.

The experiments given below show that an Eck fistula dog with a bile fistula puts out much less bile pigment than a simple bile fistula.

EXPERIMENTAL OBSERVATIONS

The general technique of the bile fistula experiments is exactly the same as described in an earlier contribution (4). The routine care of animals and methods of collection and analysis of bile have also been described in detail. The Eck fistula operation used here was devised by Jeger (5), and consists of a lateral anastomosis between the portal and vena cava by means of special clamps. It is much like a simple entero-enterostomy done with the usual spring clamps. The portal vein is then ligated above the fistula as close to the liver as possible. These Eck fistula dogs will live a normal existence in almost every respect if kept on a limited diet. Bones and milk are important elements of this diet, and the calcium may be the most important factor. At times these dogs develop peculiar intoxications which may be fatal or at times may be warded off by the administration of calcium salts. Exercise is very important for these animals. The best results have been attained when an Eck fistula was performed first, followed after weeks or months by the bile fistula operation.

Eck fistula and bile fistula

Dog 16–15. Consult tables 31, 32 and 33.
September 13, 1915. Eck fistula operation, weight 23.5 pounds.
September 25. Dog at lowest weight, 20.5 pounds.
February 17, 1916. Simple bile fistula, weight 23.5 pounds.
Common bile duct doubly ligated but not cut. Hemoglobin 101 per cent.
February 21. Dog normal, weight 23 pounds. Hemoglobin 98 per cent.
March 3. Dog active and normal. See table 31.

March 24. Dog active and normal. Weight 20.3 pounds. Hemoglobin 51 per cent.

April 3. Dog active and normal. Weight 21.3 pounds. Hemoglobin 38 per cent.

April 10. Dog active and normal. Weight 20.8 pounds. Hemoglobin 41 per cent.

April 17. Dog active and normal. Weight 21.8 pounds. Hemoglobin 49 per cent.

April 24. Dog active and normal. See table 32.

June 19. Dog active and normal. Weight 20 pounds. Hemoglobin 78 per cent.

July 5. Dog active and normal. Weight 20.8 pounds. Hemoglobin 98 per cent. Red blood cells 6,332,000.

During summer dog was in good condition, active and strong. Usual routine collection.

September 5. Dog active and normal; usual routine. See table 33.

December 1, 1916. Dog continues in the usual condition.

TABLE 31

Eck fistula, bile fistula and bile secretion

Dog 16-15*

DATE	BILE								WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams					Bread, bone and milk diet
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.		
1916									*lbs.*	
March 3	8	19	16	43	1.0	3.9	4.0	8.9	21.5	Urine contains trace of bile pigment
March 4				52				14.0	21.8	
March 6	19	14	15	38	3.2	5.4	6.4	15.0	21.5	
March 7	12	14	15	41	2.7	3.5	4.1	10.3	21.5	Hemoglobin 62 per cent
March 8	11	12	12	35	4.4	4.8	5.2	14.4	21.8	Stools contain no stercobilin
March 9	15	15	13	43	4.4	4.4	4.1	12.9	22.0	
March 10	13	10	10	33	4.1	4.7	6.8	15.6	22.0	
March 11				40				11.6	21.8	
March 13	7	9	7	23	1.6	2.2	2.3	6.1	22.3	Hemoglobin 45 per cent
March 14	14	18	17	49	3.2	4.0	2.8	10.0	22.0	
Average.....				40				11.8	21.8	

* Eck fistula operation September 13, 1915, weight 23.5 pounds. Bile fistula operation February 17, 1916, weight 23.5 pounds.

TABLE 32

Eck fistula, bile fistula and bile secretion

Dog 16-15*

DATE	BILE								WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams					Bread, bone and milk diet
	1-2 hrs.	3-4 hrs	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.		
1916									lbs.	
April 24	45	19	20	84	2.3	1.3	1.8	5.4	22.0	Hemoglobin 69 per cent
April 25	23	15	15	53	3.1	3.4	1.3	7.8	22.1	
April 26	20	17	18	55	1.8	2.3	2.0	6.1	22.3	
April 27	14	14	22	50	2.2	2.8	2.0	7.0	22.5	
April 28	23	17	19	59	2.6	2.3	2.1	7.0	22.4	
April 29				36				8.8	22.5	
May 1	21	16	18	55	4.9	2.3	3.1	10.3	22.0	Hemoglobin 58 per cent
May 4	9	4	8	21	3.1	2.4	1.8	7.3	22.0	
May 5	11	16	12	39	1.2	2.2	1.9	5.3	22.3	
May 6				44				5.0	22.0	
May 8	23	20	19	62	2.6	1.4	1.3	5.3	21.5	Hemoglobin 71 per cent
May 9	15	9	6	30	0.9	0.8	0.5	2.2	21.8	
May 10	11	18	18	47	1.5	4.0	2.8	8.3	22.0	
May 11	20	6	8	34	1.0	0.6	0.4	2.0	22.5	
May 12	14	5	8	27	1.0	0.3	0.6	1.9	22.5	
May 13				28				3.8	22.5	
May 15	22	11	17	50	3.5	2.4	2.7	8.6	22.0	Hemoglobin 79 per cent
May 16	7	13	10	30	0.6	1.2	0.7	2.5	22.1	
May 17	7	8	7	22	0.4	0.6	0.5	1.5	22.8	
May 18	19	7	6	32	2.6	1.0	0.4	4.0	22.3	
May 19	12	8	13	33	2.2	1.6	3.2	7.0	22.3	
May 20				30				6.8	22.0	
May 22	22	12	8	42	4.0	1.4	0.5	5.9	21.8	Hemoglobin 67 per cent
May 23	6	6	7	19	0.4	0.7	1.0	2.1	21.8	
May 24	15	5	5	25	3.4	0.4	0.7	4.5	22.0	
May 25	12	7	5	24	4.9	2.2	0.3	7.4	21.8	
May 26	11	6	3	20	2.9	1.8	0.4	5.1	21.8	
May 27				20				5.4	21.8	
May 29	18	9	6	33	7.2	2.8	1.7	11.7	21.5	Hemoglobin 64 per cent
May 30	10	5	11	26	1.1	0.7	2.0	3.8	21.3	
June 3				70				8.2	21.0	
June 5	26	18	12	56	2.4	2.0	0.8	5.2	21.0	Hemoglobin 74 per cent
Average.....				39				5.7	22.0	

* Eck fistula operation September 13, 1915, weight 23.5 pounds. Bile fistula operation February 17, 1916, weight 23 5 pounds. Urine free from bile pigments.

TABLE 33

Eck fistula, bile fistula and bile secretion

Dog 16-15*

DATE	BILE								WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams					Bread, bone and milk diet
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.		
1916									*lbs.*	
September 5	17	10	11	38	1.9	2.3	2.9	7.1	22.0	Stools contain no stercobilin
September 6	10	8	4	22	2.0	1.6	0.7	4.3	22.5	
September 7	13	6	4	23	2.0	0.8	0.5	3.3	22.3	
September 8	11	14	10	35	3.2	3.5	2.0	8.7	22.5	
September 11	7	2	7	16	4.5	1.5	3.9	9.9	22.1	
September 12	7	5	4	16	1.2	1.0	0.6	2.8	22.0	R. B. C. 8,728,000. Hb. 103 per cent
September 13	8	6	4	18	2.5	3.2	1.9	7.6	22.5	
September 14	4	9	10	23	1.4	4.0	2.9	8.9	21.8	
September 15	8	9	8	25	0.9	1.0	0.9	2.8	22.0	
September 16				24				14.4	22.5	
September 18	6	6	6	18	2.8	6.2	4.6	13.6	22.1	
September 19	16	7	3	26	3.6	1.2	0.2	5.0	22.0	Stools contain a trace of stercobilin
September 20	8	7	3	18	5.0	6.4	1.4	12.8	22.5	R. B. C. 7,408,000. Hemoglobin 98 per cent
September 21	5	2	4	11	1.2	0.4	1.7	3.3	22.0	
Average.....				22				7.5	22.2	

* Eck fistula operation September 13, 1915, weight 23.5 lbs. Bile fistula operation February 17, 1916, weight 23.5 pounds. Urine at no time contained bile pigments.

Simple bile fistula

Dog 16–146. Small, active, pugnacious, mongrel male.
April 21, 1916. Weight 18.8 pounds. Hemoglobin 114 per cent.
Simple bile fistula. Common duct ligated and cut.
May 2. Weight 17.5 pounds, hemoglobin 97 per cent. Active and normal.
May 8. Weight 17.5 pounds, hemoglobin 101 per cent. Active and normal.
May 15. See table 34.
During this time dog had no jaundice, and urine was free from bile pigments.
October 4. Dog is in good condition.

This dog (16-146) acts as control for dog 16-15 with bile and Eck fistula. Both dogs were pugnacious, active adult males of the same build but not the same breed. The control is smaller, 17 pounds as compared with 22 pounds, but puts out slightly more bile than the Eck fistula, 41 cc. as compared with the average of 39 cc. The difference in *bile pigment output* is remarkable, 17.7 mgm. for the normal as contrasted with 5.7 mgm., the average for the Eck fistula.

TABLE 34

Simple bile fistula, control for Eck fistula. Contrast tables 32 and 33

Dog 16-146*

DATE	BILE								WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams					
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.		Mixed diet
1916									*lbs.*	
May 15	9	13	12	34	5.2	8.2	4.8	18.2	17.3	Hemoglobin 98 per cent
May 16	14	15	14	43	4.7	6.4	5.0	16.1	17.5	
May 17	12	14	14	40	4.6	5.6	5.0	15.2	17.8	
May 18	8	13	19	40	3.1	5.0	5.6	13.7	18.0	
May 19	9	15	17	41	5.1	10.0	6.8	21.9	17.8	
May 20				40				17.2	18.0	
May 22	14	15	20	49	5.4	6.0	6.8	18.2	17.8	Hemoglobin 95 per cent
May 23	8	13	15	36	4.6	6.4	6.0	17.0	18.0	
May 24	18	18	17	53	6.4	6.0	5.4	17.8	18.3	
May 25	8	17	18	43	4.0	6.8	5.7	16.5	18.1	
May 26	16	19	18	53	6.1	7.6	6.0	19.7	18.3	
May 27				32				19.4	18.0	
May 29	14	16	15	45	6.2	7.2	5.7	19.1	17.8	Hemoglobin 96 per cent
May 30	9	7	8	24	4.9	6.1	7.4	18.4	18.0	
Average.....				41				17.7	17.1	

* Bile fistula operation April 21, 1916. Weight 18.8 pounds. Urine free from bile pigment.

Eck fistula and bile fistula

Dog 15-15. Strong white bull dog, female.

May 13, 1915. Weight 32 pounds, hemoglobin 91 per cent. Red blood cells 5,376,000.

Operation, Eck fistula and bile fistula done at same time. Common duct ligated and cut.

May 25. Dog in good condition, weight 30.3 pounds, hemoglobin 90 per cent. Red blood cells, 5,248,000.

May 31. See table 35.

July 15, 1915. Dog intoxicated and was sacrificed.

Autopsy. The Eck fistula is wide open, and the portal vein above it is obliterated. Bile fistula in good condition, common duct obliterated at its entrance into duodenum. Other findings not germain to this e periment.

TABLE 35

Eck fistula, bile fistula and bile secretion

Dog 15–15*

DATE	BILE								WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams					Diet†
	1–2 hrs.	3–4 hrs.	5–6 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	Total 6 hrs.		
1915									*lbs.*	
May 31	15	15	16	46	3.1	3.4	2.9	9.4	29.5	May 13 R. B. C. 5,376,000. Hemoglobin 91 per cent
June 1	18	22	15	55	3.5	4.3	2.6	10.4	30.0	R. B. C. 5,200,000. Hemoglobin 92 per cent
June 2	13	11	16	40	1.8	2.0	2.8	6.6	29.5	
June 3	15	15	22	52	2.0	2.6	3.5	8.1	29.5	Stools contain no sterocobilin
June 7	24	22	23	69	7.5	7.2	7.3	22.0	29.3	
June 8	11	16	28	55	0.1	0.7	1.6	2.4	29.3	
June 9	20	23	18	61	3.1	6.1	5.1	14.3	30.0	
June 10	19	18	21	58	2.5	2.5	4.3	9.3	30.3	
June 11	8	10	15	33	1.3	2.7	3.6	7.6	30.0	Hemoglobin 90 per cent
June 14	29	26	16	71	2.6	3.0	2.9	8.5	29.5	Stools contain no stercobilin
Average.....				54				9.8	29.6	

* Bile fistula and Eck fistula operations May 13, 1915.

† Diet of milk, bread and bones plus 50 grams liver each day. Urine contains at most a trace of bile pigments.

Eck fistula and bile fistula

Dog 16–139. Black and white mongrel male.

March 28, 1916. Weight 32.5 pounds.

Operation, Eck fistula done as usual.

April 10. Good condition, weight 30.8 pounds, hemoglobin 87 per cent.

April 24. Good condition, weight 30.8 pounds, hemoglobin 97 per cent.

April 28. Simple *bile fistula* operation.
May 16. Weight 28.8 pounds, hemoglobin 98 per cent.
May 17. See table 36.
June 12. Weight 29.5 pounds, hemoglobin 88 per cent, good condition.
July 5. Distemper developed.
July 8. Death.

Autopsy. Eck fistula is partly occluded except for three small openings, probably due to organized thrombi. The portal vein above the fistula is completely occluded. The bile passages are pale and smooth. The bile fistula is perfect, and bile is completely excluded from the duodenum. Kidneys show miliary abscesses.

TABLE 36

Eck fistula, bile fistula and bile secretion

Dog 16–139*

DATE	BILE								WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams					
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.		Bread, bone and milk diet
1916									*lbs.*	
May 17	4	10	8	22	2.2	4.3	2.6	9.1	29.0	Urine constantly free from bile pigments
May 18	8	8	10	26	3.6	3.6	4.0	11.2	28.5	
May 19	9	9	9	27	4.9	4.0	2.8	11.7	28.3	
May 20				22				14.9	28.3	
May 22	11	9	10	30	5.4	4.2	4.6	14.2	28.5	Hemoglobin 96 per cent
May 23	6	5	9	23	4.0	2.5	4.6	11.1	28.8	
May 24	9	11	10	30	2.8	4.4	3.4	10.6	29.0	
May 25	11	13	14	38	7.6	6.4	6.4	20.4	28.8	
May 26	7	9	15	31	5.7	5.2	6.8	17.7	29.1	
May 27				60				18.8	29.3	
May 29	12	10	11	33	8.7	6.0	4.8	19.5	28.8	Hemoglobin 86 per cent
May 30	8	9	8	25	2.3	3.4	3.6	9.3	29.0	
May 31	8	12	11	31	5.8	6.4	5.0	17.2	29.0	
Average.....				31				14.2	28.8	

* Eck fistula operation March 28, 1916, weight 32.5 pounds. Bile fistula operation April 28, 1916, weight 31.3 pounds.

Dog 15–18. Strong, adult, Airdale female.

March 26, 1915. Weight 30 pounds: hemoglobin 110 per cent. Red blood cells, 6,340,000.

Eck fistula done as usual. *Splenectomy* and simple *bile fistula*.

Common duct ligated and cut.

March 31. Weight 29.8 pounds: hemoglobin 110 per cent. Red blood cells, 6,480,000.

April 13. See table 37.

May 4. Weight 25 pounds; hemoglobin 104 per cent; muscular tremors and intoxication.

Autopsy. The Eck fistula is wide open, and the portal vein above it obliterated. Bile is completely excluded from the duodenum, and the bile passages are smooth and pale.

The other findings do not concern this experiment.

For an accurate control of this experiment refer to Table D and many others in a preceding paper, No. III of this series, which gives the bile pigment output of a simple bile fistula plus a splenectomy.

TABLE 37

Eck fistula, bile fistula and splenectomy. Bile secretion

Dog 15–18*

DATE	BILE								WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams					Diet†
	1–2 hrs.	3–4 hrs.	5–6 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	Total 6 hrs.		
1915									*lbs.*	
April 13	20	18	15	53	4.9	4.8	4.5	14.2	29.0	March 31, R. B. C. 6,480,000; hemoglobin 110 per cent
April 14	15	12	15	42	4.4	4.0	6.3	14.7	29.0	
April 15	23	18	21	62	7.1	5.3	7.1	19.5	29.0	Stools contain no stercobilin
April 16	27	18	19	64	9.2	5.5	5.5	20.2	28.5	
April 19	22	23	15	60	9.6	7.0	5.0	21.6	29.0	
April 20	20	22	26	68	6.9	6.7	8.7	22.3	38.8	R. B. C. 6,144,000; hemoglobin 103 per cent
April 21	22	23	20	65	7.9	8.5	6.7	23.1	28.5	
April 22	15	13	17	45	6.5	7.2	8.5	22.2	28.0	
April 23	22	19	21	62	5.9	7.4	7.5	20.8	28.5	
April 26	16	15	19	50	1.8	1.7	2.6	6.1	28.0	R. B. C. 5,367,000; hemoglobin 103 per cent
April 27	26	33	42	101	4.0	5.9	5.6	15.5	28.0	
Average.....				61				18.2	28.5	

* Eck fistula, bile fistula and splenectomy operations March 26, 1916.

† Diet of milk, bread and bones plus 50 grams cooked liver each day. Urine contains at most a trace of bile pigments.

Simple bile fistula

Dog 16–5. Strong, white bull dog, male.

September 1, 1915. Weight 34.5 pounds, hemoglobin 104 per cent. Red blood cells, 5,696,000.

Simple bile fistula, common duct ligated and cut.

October 22. Weight 29.8 pounds, hemoglobin 93 per cent. Red blood cells 6,360,000. Dog is in good condition.

November 11. Weight 29 pounds, hemoglobin 111 per cent; condition is good.

January 20. See table 38.

February 5. Death from intoxication.

Autopsy. Bile fistula is perfect, and the bile passages are pale and smooth. Liver cells are slightly pigmented.

This dog serves as a control for tables 35 and 36. Other similar experiments have been tabulated in the preceding papers.

TABLE 38

Simple bile fistula, control for Eck fistula. Contrast tables 35 and 36

Dog 16–5*

DATE	BILE								WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams					Mixed diet
	1–2 hrs.	3–4 hrs.	5–6 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	Total 6 hrs.		
1916									*lbs.*	
January 20	25	21	29	75	9.0	6.1	7.8	22.9	30.0	
January 21	22	23	21	66	7.0	8.2	8.4	23.6	31.0	Stools contain no stercobilin
January 22				32				36.5	30.5	
January 24	23	18	21	62	17.0	12.1	14.4	43.5	30.5	Hemoglobin 90 per cent
January 25	21	15	22	58	13.3	11.2	12.8	37.3	30.0	
January 26	17	22	19	58	7.2	11.4	10.	29.4	30.5	
January 27	16	17	19	52	7.9	8.0	9.4	25.3	29.5	
January 28	43	17	20	80	14.4	8.4	9.4	32.2	30.3	
January 29				54				26.8	31.0	
January 31	22	20	24	66	12.4	12.7	14.0	39.1	30.5	Hemoglobin 91 per cent
Average.....				60				31.7	30.4	

* Bile fistula operation September 1, 1915. Urine contains trace of bile pigments.

TABLE 39

Simple bile fistu'a. Control for Eck fistula diet

Dog 15–22

DATE	BILE								WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams					Bread, bone and milk diet
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.		
1915									*lbs.*	
November 1	15	16	18	49	13.5	15.1	16.0	44.6	31.5	Stools contain stercobilin
November 3	16	18	20	54	14.4	17.1	16.7	48.2	31.8	October 25, hemoglobin 123 per cent
November 4	17	23	20	60	16.0	19.1	16.3	51.4	31.8	
November 5	15	20	19	54	13.5	13.5	13.3	40.3	32.0	
November 6				45				41.5	31.8	
November 8	17	20	24	61	11.9	13.6	12.7	38.2	31.3	
November 9	19	23	21	63	13.3	14.2	12.8	40.3	31.3	
November 10	26	21	25	72	8.0	9.2	1 .4	29.6	31.3	
November 11	28	26	25	79	11.1	11.4	10.5	33.0	31.5	Hemoglobin 121 per cent
November 12	16	23	22	61	12.6	13.2	12.6	38.4	31.3	
November 13				55				42.5	31.3	
November 15	14	23	21	58	14.5	13.2	14.4	42.1	31.0	
November 16	18	23	20	61	15.0	18.2	12.6	45.8	31.5	
November 17	20	19	23	62	16.7	15.8	15.2	47.7	31.3	
Average.....				59				41.6	31.4	

Urine contains at most a trace of bile pigments.

DISCUSSION

The experiments tabulated above are only a few out of a considerable number which have been performed. It is somewhat difficult to produce a successful Eck fistula in a dog and at the same time or later a bile fistula without impairing seriously the general condition of the animal. Both Eck fistula dogs as well as bile fistula dogs are subject to peculiar types of intoxication which are familiar to laboratory workers. It is not surprising, therefore, that dogs with combined Eck and bile fistulae are also subject to obscure and troublesome disturbances. The observations given above show the output of bile and bile pigment in dogs with bile and Eck fistulae during normal periods or at

least periods of normal activity, appetite, digestion and weight. Dogs like No. 16–15, tables 31–33, are of particular interest, as this animal has been under observation with an Eck fistula for over a year in apparent perfect health.

All our experiments with combined bile and Eck fistulae (nine in all) are quite in accord, and these experiments here reported are fair examples. In all experiments there is definite evidence of a fall in bile pigment output below a normal mean—a fall sometimes to one-third normal, or perhaps at times only to three-fourths of normal, but always a fall in the average output of bile pigments per unit period under uniform conditions. There are the same daily and hourly fluctuations in bile pigment output, which we cannot at present explain. These fluctuations are present in the simple bile fistula as well as in the combined bile and Eck fistula.

It must be noted that Eck fistula dogs will do best on a diet of bread, milk and bones. This diet, rich in carbohydrates, gives a high level of bile pigment secretion, and table 39 gives a review of this factor, which was emphasized in an earlier communication (6). We must keep in mind the fact that a simple bile fistula dog on this Eck fistula diet will put out on the average more than 1 mgm. of bile pigment per pound body weight per unit period of six hours.

Splenectomy added to a combined bile and Eck fistula does not modify the picture nor does it change the general output of bile pigments. The particular experiment tabulated (table 37) gives a bile pigment secretion somewhat above the usual average, but this is not constant, and we believe has no significance.

May not the deviation of the digestion products from the portal vein into the vena cava and so around the liver be responsible for this drop in bile pigment secretion? We are unable to answer this question at present. An explanation on this basis would assume that some pre-pigment substance might be built up by the liver using materials brought to it by the portal vein. Such a substance might be used in the body or thrown out in the bile pigment depending on supply and demand. It might be assumed that with an Eck fistula this material was not as directly available, and the pigment formation correspondingly decreased. It is at least possible that some such factor is a part of this equation.

It is important to know whether the Eck fistula liver can eliminate promptly and in usual amounts the pigment radicle when hemoglobin is injected intravenously. Does the Eck fistula liver eliminate the same

amount of bile pigment following an injection of hemoglobin? This question may be answered in the affirmative, but the detailed experiments will be given in a later publication. So this low pigment output can not be explained by a faulty pigment elimination by the liver. All the evidence points to faulty or abnormally low pigment formation, and this may not be limited to bile pigments. There are experiments which suggest that the hemoglobin may be involved in this incomplete pigment formation (refer to table 32 and the abstract history of dog 16–15 given above).

What is to be said about the life cycle of the red cell as calculated by the end products of hemoglobin disintegration? The pigment products are obviously much less in the Eck fistula dog. Must we assume that the abnormal environment of the Eck fistula dog tends to favor immortality among the short-lived red cells? This may suggest that the common method of computing the life of a red cell from the amount of its end products (?) is open to error. There are at least two unknowns in this equation.

We may say that the autopsy material gives no definite anatomical evidence of abnormality in the hematopoietic system. The simple bile fistula and the combined bile and Eck fistula show the same picture in the bone marrow, spleen and lymph glands. The only anatomical evidence is to be found in the atrophied Eck fistula liver which shows central fatty degeneration and atrophy. There is definite evidence that the Eck fistula liver may be functionally inefficient (2). We are inclined to explain the low bile pigment output in the Eck fistula as due to lowered functional capacity of the liver. We admit that other factors may be concerned, for example, the deviation of digestion products round the Eck fistula liver, supposing that some pre-pigment substance may be elaborated by the liver from such digestion products.

Whatever the correct explanation, the fact remains that the Eck fistula liver puts out much less bile pigment than normal under uniform conditions. This means more evidence that bile pigment can be formed independently of red blood cell or hemoglobin disintegration, which plays a part (perhaps a small part) in the secretion of bile pigments by the liver.

SUMMARY

An Eck fistula allows the portal blood to pass directly into the vena cava and excludes it from the liver.

The Eck fistula liver is atrophic with central fatty degeneration, and shows evidence of subnormal functional capacity.

The Eck fistula liver secretes much less bile pigment than the normal liver—one-third to three-fourths of normal or even less.

Splenectomy added to the combined bile and Eck fistula does not essentially modify the result.

Simple bile fistula and the combined bile and Eck fistula dogs show the same histological picture in the hematopoietic system.

This means that bile pigment formation is in part dependent upon liver function—in other words not solely dependent upon red cell disintegration. It is at least possible that the production of other body pigments (e.g., hemoglobin) may depend in part upon the constructive capacity of the liver.

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THE ORIGIN OF THE PROTEOLYTIC FERMENTS

II. Concerning the Character of Certain Ferments

LeROY H. SLOAN

From the Laboratory of Pharmacology, Northwestern University Medical School

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In a previous article (1) results were presented bearing on the specificity and character of the enzyme in the Abderhalden reaction and conclusions stated as follows:

1. There is an increased proteolytic activity of blood serum during pregnancy.

2. This increased activity is probably in the main due to an increase in the polyvalent ferments.

3. Normal blood serum has a weak non-specific proteolytic action.

4. The dialysis method of Abderhalden or the method of Williams and Pearce does not suffice to demonstrate a ferment developed during pregnancy acting only upon placental protein.

5. Many advanced pathological conditions give a positive reaction within the time specified for the Abderhalden test.

6. At present the Abderhalden test is quantitative, not qualitative.

Most of the work published since on the problem above stated, confirms the conclusions as given. Reviews of the literature dealing with the Abderhalden and associated reactions have recently been given by Hulton (2), Bronfenbrenner (3) and Wallis (4). Only such literature as bears directly upon the problem is noted here.

Recently Van Slyke, Vinograd-Villchur and Losee (5) using the familiar amino nitrogen method of Van Slyke, reported that normal blood serum digests placental tissue with practically the same quantitative result as the serum of pregnant women. They conclude, therefore, that in pregnancy there is no definite increase of proteolytic ferments in the serum. Consequently they question the reliability of the Abderhalden method for the diagnosis of pregnancy and also question the theory advanced by Abderhalden to explain this reaction (6). The theory is, briefly, that during gestation chorionic cells from the villi pass into the blood stream of the mother. In response to the entrance

of these foreign (?) cells ferments are developed which are "protective" in character. That chorionic villi are found in the blood stream is an established fact. By some the albuminuria of pregnancy is attributed to such foreign protein. Early in the development of the Abderhalden method it was maintained, especially by the originator of the test and his associates, that such ferments were not only proteolytic but also specific. The vast amount of work done in connection with this particular phase of specificity has not served to support the contention, however. The early work upon which the test is based is thoroughly reviewed by Lake (7).

The results of Van Slyke et al. do not coincide with the generally accepted view that in pregnancy there is developed a definite increase in the concentration or strength of proteolytic ferments. Neither do they coincide with the results which I obtained and reported in the paper mentioned. Because of the apparent discrepancy in the results and the bearing which the above has on the origin of the proteolytic ferments, I repeated previous work and studied more carefully the theory of the production of the ferments in gestation.

It was found previously that the blood serum of pregnant dogs digests human placenta with the same ease as the serum of pregnant women. Consequently in this work dogs were used exclusively and the following series of experiments carried out in the order mentioned.

Series I. The action of the blood serum of normal dogs upon human placenta.

Series II. The action of the blood serum of pregnant dogs on human placenta.

Series III. The action of the blood serum of dogs injected with placental suspension.

Series IV. The action of the serum of dogs injected with split products of pepsin-trypsin digestion of human placenta.

Series V. The action of the serum of pregnant dogs injected with placental suspension.

Series VI. The effect of feeding on the amino acid content and the ferment strength of the blood serum using placenta as the substrate

SERIES I. THE ACTION OF THE BLOOD SERUM OF NORMAL DOGS UPON HUMAN PLACENTA

Technic. Dogs were starved from eighteen to twenty-four hours. Blood was withdrawn from the external jugular vein under sterile precautions in a sterile syringe. It was at once put into a sterile test-tube

with cotton stopper through which passed a safety pin. The safety pin prevented the stopper from being drawn down into the test-tube during the period of centrifuging, which was begun immediately and continued for ten to fifteen minutes at high speed. The serum was allowed to remain in contact with the solid parts of the blood until needed and was then withdrawn in a sterile pipette. The serum thus prepared was absolutely corpuscle-free in the majority of cases and its ferment strength not impaired when judged by the present method for estimating proteolytic ferments and diastatic ferments. We believe, possibly without sufficient reason, that serum so prepared more nearly approaches the normal ferment-bearing portion of the blood. Those changes which certainly must take place when blood is allowed to clot and slowly to express its serum are minimized.

Two cubic centimeters of serum was incubated with 0.5 gram of moist placenta or the equivalent of dried placenta prepared in the following manner: The placenta was obtained through the kindness of the staff of Cook County Hospital, brought to the laboratory, ground, washed and then floated off on water from a deep beaker into a flat glass dish. In this way we could inspect every particle of placental material and could exclude all tissue elements aside from the chorionic villi. This method is the only one which has proven adequate to the preparation of a white, specific substrate. Further details of the treatment are given in the previous article (loc. cit). In several of the experiments dried placenta was used. This is better suited to the method if in finely divided form and if the serum is agitated with the substrate at the beginning of incubation in order to insure a better admixture. All incubation mixtures contained 0.5 cc. to 1.0 cc. of toluol. Controls on 2 cc. of serum without substrate were run on each experiment. At various intervals the water, toluol, dialyzed iron and nitrite solution were tested after incubation and the amino nitrogen determined. Correction for materials averaged 0.20 cc. of gas. After incubation the specimens of serum and serum plus substrate were treated as given by Van Slyke et al. (loc. cit.). The disadvantages of the Van Slyke method when applied to the experiments below are apparent. Blood serum is incubated with a non-soluble, complex protein more or less finely divided, sometimes moist and always in a test-tube where uniform intermixture of serum and substrate is impossible. Again, dialysis of the products of digestion is not possible. While, therefore, the method gives very accurate results when applied to duplicate samples of the serum itself, satisfactory determinations are not always obtained when

serum and substrate are incubated. This factor has made repetition of experiments necessary in not a few instances. The use of dry substrate does not overcome the uncertainty, though it does lessen it. From this it is evident that satisfactory duplicates must be run on every incubation mixture. In several instances triplicate tubes have been measured. It is possible that the reason for the uncertainty of the reaction is explained by the conclusion of Frankel (8) that ". . . . comparable results in proteolysis studies are to be obtained only when the substrates are in solution . . . ." Such discrepancy is not found when adequately tested dialyzing experiments are carefully carried out.

Experimental results. The results are calculated in milligrams of amino nitrogen in 2 cc. of serum with or without placenta.

TABLE 1

The action of the blood serum of normal dogs upon human placenta

DOG	MILLIGRAMS OF AMINO NITROGEN IN			INCUBATION TIME IN HOURS
	2 cc. serum and 0.5 grams placenta	2 cc. serum	Due to ferment action	
1	0.112	0.056	0.056	20
2	0.133	0.061	0.072	23
3	0.194	0.149	0.045	23
4	0.160	0.100	0.060	23
5	0.189	0.156	0.033	25
6	0.220	0.110	0.110	20
7	0.179	0.124	0.055	20
8	0.136	0.081	0.055	22
9	0.122	0.058	0.064	22
10	0.101	0.067	0.034	20
11	0.084	0.034	0.050	20
Average	0.148	0.090	0.057	

The degree of digestion to be expected in a normal dog under the above conditions I believe to be fairly represented by the average figure given. For very obvious reasons there can be no absolute figure. But in selecting an average, note must be taken of the following: dogs differ in the concentration of amino acids in the blood under the same conditions; there is a rather wide variation in the ferment strength of the serum. While we have included no experiments, we have also found that the amino acids do not remain constant from day to day in the same dog, nor do the ferments. Unknown factors modify their

concentration. In a few instances dogs have shown almost no ferment when a single specimen was examined but in no case was a serum found which did not contain substances giving an amino acid reaction.

SERIES II. THE ACTION OF THE BLOOD SERUM OF PREGNANT DOGS ON HUMAN PLACENTA

Technic. To compare the action of the enzymes normally present with those present during pregnancy four dogs were tested, all of which were in fine condition, and found pregnant while in the laboratory. The following reactions are typical and were performed at a time when the dogs were supposedly at practically a maximum enzyme height, i.e., a few weeks (2 to 3) previous to labor (Sloan, loc. cit.).

TABLE 2

The action of the blood serum of pregnant dogs on human placenta

DOG	MILLIGRAMS AMINO N DUE TO FERMENT ACTION	INCUBATION TIME IN HOURS
13	0.039*	24
14	0.008*	20
15	0.050*	20
27	0.062*	20

* Abderhalden dialysis tests were positive in each case.

The results agree with those of Van Slyke et al. By this method a greater concentration or action of pregnant serum over non-pregnant serum cannot be demonstrated. And yet on the other hand each of the dogs gave a *good Abderhalden test.* This the non-pregnant dog if in normal condition does not do in eighteen to twenty hours. The conclusion that the ferments of the blood are *not* increased during pregnancy must be limited *to this method.* Here by quantitative measurement the amino acid concentration in a digestion mixture is determined. In the dialysis test we measure the reaction of an indicator to substances, produced by digestion, which have passed through a semipermeable membrane out into the surrounding water. Exactly what substances react with ninhydrin has not been shown. Certainly the result is not dependent upon the amino acids entirely but is probably much influenced by the intermediate products. The micro amino method is limited to one type of product which at the present time is considered to be the ultimate unit of the protein molecule. Under the conditions of incubation the dialysis method gives opportunity for a

more comprehensive reaction than does the Van Slyke. Van Slyke continued the digestion of soluble intermediate products by an acid hydrolysis but could find practically no difference in the end result. He does state, however, that pregnant sera produce an average digestion slightly greater than the normal. The variation is so marked between individual specimens, however, that the test has no practical application to pregnancy. It is important to note that there is a slight difference between the pregnant and the normal for in so large a series as that reported by Van Slyke et al. this difference cannot be attributed to experimental error which might be the case in a short series. Thar and Kotschneff (quoted by Van Slyke) report that on incubating normal and pregnant sera with placenta in dialyzing thimbles and determining the amino acids in the dialysate they obtained negative results with normal but positive results with pregnant sera. The ferment was not specific, however. When incubation experiments without dialysis were carried out there was noted no difference between pregnant and normal sera. I believe, therefore, that the method of Van Slyke is not an adequate one for testing the ferment of pregnancy in the way mentioned above and that the original dialysis technic when carefully and repeatedly controlled is of much greater value.

SERIES III. THE ACTION OF BLOOD SERUM OF DOGS INJECTED WITH PLACENTAL SUSPENSION

As mentioned earlier in this paper, the dog produces an enzyme during pregnancy which will digest human placenta within a period of eighteen to twenty hours. This digestion can be demonstrated by the dialysis technic. Furthermore, the *normal* dogs' serum does not have this power except the interval be continued for six to eight hours.

Now, if the theory presented by Abderhalden to explain this increase in proteolytic power is correct, we should expect the dog to develop a ferment when injected with chorionic cells. That the dog is not more widely used for immunizing purposes depends upon the fact that dogs are not so easily sensitized or immunized as the smaller rabbit or guinea pig. In this particular instance we should not expect such a condition to exist. Moreover, if the *method of introduction* of chorionic cells approximates the supposed method of entrance during gestation we would have much more reason to expect the generation of a ferment than if an entirely different procedure were chosen such as intramuscular injection.

Technic. Five grams of placenta which had been prepared according to the method outlined earlier was ground under sterile conditions in a small amount of salt solution until in very finely divided form. The undivided portion (about 0.5 grams) was discarded and the whole suspended in salt solution to which a little bicarbonate of soda had been added. This suspension was decidedly opalescent and very little settling-out occurred. The suspension was shaken before using, however. Four dogs were injected intraperitoneally with from 12 to 30 cc. of the suspension. Following this at intervals of three days the dogs received four to five *intravenous* doses of increasing amount (2 to 20 cc.). Blood was withdrawn and its ferment action determined as given in the table.

TABLE 3

The action of blood serum from dogs injected with placental suspension

DOG	SEX	NUMBER OF DAYS AFTER LAST INJECTION	MGM. AMINO N DUE TO FERMENTS	ABDERHALDEN TESTS	INCUBATION TIME IN HOURS
17	♀	Before	0.056		22
		10	0.082		21
		13	0.064	+ −	20
		23	0.076		20
Average...	.....	.....	0.069		
18	♂	Before	0.064		22
		10	0.055		21
		13	0.059	+ − −	20
Average...	.....	.....	0.059		
19	♀	13	0.073		20
		21	0.109		20
		7	0.059		21
		13	0.022		20
		23	0.082	−	21
Average...	.....	.....	0.069		
20	♀	7	0.092	−	24
		12	0.031	−	23
		26	0.066		23
		33	0.094		20
Average...	.....	.....	0.070		
General average		.....	0.066		

* The Abderhalden reactions are interpreted as follows: + − questionable; − negative.

Discussion. It will be noted that the average of the results using the Van Slyke method is higher in each case than the normal given in table 1. Does this indicate an enzyme liberation? While I think that it *indicates* such, it does nothing more, since the general average is so slightly increased over the normal (0.009). Of course, a slight increase of amino acids may mean considerable increase in the total digestive products. But on the other hand when we apply the dialysis method we find no apparent increase. Consequently, the slight increase noted here is not sufficient to justify a conclusion that a proteolytic antibody or enzyme has been generated. On the other hand had the general average been 0.100 or thereabouts such a conclusion would have been justified. The results confirm those of Hulton (9) who reported experiments on the injection of human placenta into dogs subsequent to the beginning of this work. In her experiments, however, dogs were tested but once and after intraperitoneal and intramuscular injection of emulsion. No increase in ferments was noted using the Kjeldahl method for determining the degree of digestion. However, the mode of injection of the emulsion does not test the underlying theory of the pregnancy reaction as advanced by Abderhalden and his associates. It does not introduce chorionic cells directly into the circulation and in so far does not approach the supposed mechanism of production of protective ferments during gestation. Of course, antibodies are produced to many foreign materials by intraperitoneal and intramuscular introduction. But in so doing it is a question whether we introduce the foreign protein as such into the blood or whether it is not the products of the action of living proteolytic ferments which stimulate the formation of these antibodies. In the experiments above, dogs received intraperitoneal injections purely in the hope that they might the better be prepared for the intravenous injection. At the present time no preliminary dose would be given. Again, the experiments were carried over a considerable interval and the animals tested several times. Of more importance is the fact that small doses were used. Such a method approximates to a much greater degree the normal mechanism of entrance of villi and consequently more nearly approaches theoretical conditions. The results, therefore, more adequately and completely disprove the theory given by Abderhalden to explain the reaction. To further confirm the results of the somewhat uncertain Van Slyke, experiments using the dialysis method were carried out, all of which were negative. We used coagulated material for injection and to this extent theoretical conditions were disregarded. We should expect animals to react to this material if able to react to living cells.

On the other hand Wallis (loc. cit.) reports, though not in detail, experiments in which he claimed to show that heating the extract of fresh placenta to 60° destroyed the power of antigen action. The recent work of Elsesser carried out with immune sera and pure proteins is of interest along this line. He concludes (10) "The experiments have moreover shown that a rabbit may react in absolute specific manner at one time although it has not done so in a previous test and does not do so in the serum obtained subsequently" . . . . "The individual rabbits also appear to differ in their reacting powers" . . . . "The serum of an immune rabbit is not constant in its reactive power over a continued period nor in the degree of specificity it exhibits." Hulton (loc. cit.) using a different method on *pure proteins* of the same sort reports no production of ferment at all. Her method was somewhat similar to that of Van Slyke. From the work so far presented we conclude that an animal must be studied over a considerable period before any adequate conception of the reaction of that animal can be obtained. Because the results obtained above are so little in excess of the general average of normal dogs and do not show a tendency to follow an antibody curve and further because dialysis tests on the same animals and at the same time were at most questionable, I feel certain that no digestive enzymes or antibodies can be produced by coagulated, non-soluble, complex placental substance when tested in the manner given.

SERIES IV. THE ACTION OF THE SERUM OF DOGS INJECTED WITH SPLIT PRODUCTS OF PLACENTAL DIGESTION UPON HUMAN PLACENTA

Five grams of coagulated placenta was digested for twenty-four hours with a moderately strong pepsin-HCl solution. At the same time a like amount was digested by trypsin. The digestion mixtures were united, brought to a boil, filtered, neutralized with bicarbonate of soda and with 0.5 gram of finely divided placenta made up to 200 cc. with normal salt. This system would approach conditions imaginable if ferments either in the blood or in the placenta acted upon chorionic villi and caused cells and breakdown products to pass into the maternal circulation, there, or more probably in the tissue cells of specialized organs, to excite accentuation of preformed ferments or the production of new. Two dogs were injected and the results are given in the following table.

TABLE 4

The action of the serum of dogs injected with split products of placental digestion upon human placenta

DATE	PROCEDURE	MILLIGRAMS OF AMINO NITROGEN DUE TO FERMENT DIGESTION		INCUBATION IN HOURS
		Dog I	Dog II	
August 29...	Withdrew blood, injected 10 cc.*	0.036		20
September 2...	Withdrew blood, injected 6 cc.†	0.039		
September 4...	Injected 7 cc.			
September 7...	Injected 15 cc.			
September 12...	Injected 30 cc.			
September 21...	Withdrew blood	0.056‡	0.034‡	20
September 31...	Withdrew blood	0.062	0.055	20
October 5...	Withdrew blood	0.034‡	0.015‡	20

* Intraperitoneal injection.

† Intravenous injections followed.

‡ On these days Abderhalden tests were run. In each case they were *double positive or greater*. The serum gave no color at all.

Here we find as in our previous experiment that there is an indication of a ferment production in the first dog. It is merely an indication and the highest result is only 0.005 beyond the average for the normal animal: *In contradistinction to the results on the injection of coagulated protein we find here that strong Abderhaldens were obtained.* This is conclusive evidence that there occurred an increase of ferments which we were neglecting when making determinations by the Van Slyke method. Here as in pregnant animals, a strongly positive test is obtained when the serum acts upon substrate in dialyzing thimbles. The products of digestion can pass into the surrounding fluid medium. But when the incubation is carried on in a test-tube and the determination of the end result is made by the amino nitrogen quantitative technic, no such production or liberation of antibody-enzymes can be detected. Theoretically the amino nitrogen procedure should be much more delicate and therefore, more dependable. In my hands such is not the case. The results above are well within the normal variations of serum action. There is no indication of a regular, steady increase of ferments after injection of foreign protein which carry digestion to a greater degree than normal. For this reason, I do not believe that the Van Slyke method will detect the ferment of pregnancy when used under the conditions as previously mentioned. On the other hand the dialysis method with proper care will show the increase found by the

majority of workers to occur during the period of gestation. Furthermore, it would seem that the theory underlying the increase of ferments during the period of intrauterine fetal life involves not so much the passage of chorionic villi into the blood stream as the passage of the digestive products of placental cells which stimulate the maternal tissue cells, possibly of special organs, to respond with a ferment of protective character. It is also possible that a weak ferment exists in the placenta itself which acts slowly upon the chorionic villi giving rise to certain substances which act as antigen.

In her recent work Hulton (loc. cit.) brought forth the suggestion that foreign protein introduced into the blood stream goes into the body cells, is hydrolyzed there, without a protein enzyme appearing in the circulating blood, concluding that hydrolysis of foreign protein is a separate action from the "fixation" of the foreign substance by the maternal body cells or the reaction of the body to these cells. The results presented above might be so interpreted but only if the interpretation be limited to the method of Van Slyke. On the other hand, based on the dialysis method, no such interpretation of the results can be made. In other words, it would seem reasonable to suppose that in the great majority of cases antibodies or enzymes are produced as a response to the injection of foreign protein and that these antibodies appear in the blood stream but in certain instances the methods are not sufficiently delicate to detect them. Hektoen and Carlson (11) stated in their work that blood cells of foreign origin when introduced into the blood stream of another animal very quickly disappeared but that antibodies were produced which could be shown to be present not only in the blood stream but also in the other circulating fluids. They state, "Our results consequently support the view that antibodies in active immunization are produced outside the circulating blood into which they find their way at the end of the latent period." This it would seem is applicable in the great majority of cases to the introduction of foreign protein. It is very probably applicable to the injection of placental protein but because the detection of ferments is more difficult than the detection of lytic agents to blood corpuscles it is less apparent under certain conditions. It would seem to us that the method of Van Slyke is not one to be used to determine the presence or absence of such antibodies. Based on the above results and those of Thar and Kotschneff and Elsesser, I believe that dialysis plays a very important rôle in the discrepancy between the so-called Abderhalden dialysis test and that of Van Slyke.

SERIES V. THE ACTION OF THE SERUM OF PREGNANT DOGS INJECTED WITH PLACENTAL SUSPENSION

Two dogs known to be pregnant were injected with placental suspension in much the same manner shown in the experiments reported above. Serum was removed in the same manner and the digestive action upon placenta determined by the Van Slyke method. Neither dog showed any increase of proteolytic ferments. The variations were not less than normal nor the limits greater. Consequently, based on this method, the conclusion is reached that the pregnant dog is no more able than the non-pregnant to mobilize or generate enzymes capable of digesting placental suspension. In this connection it is interesting to note that neither dog aborted as a result of the injections. At the conclusion of the experiment one dog was injected with the products of the action of serum (on placenta) from animals which had received intravenous placental injections. Five pups were born twenty-four hours later and the mother died at the end of about forty-eight hours. Three pups had not been delivered. The uterus had ruptured and the head of one pup protruded through the rent. We have not known of a dog dying of a ruptured uterus and present this purely as an interesting observation. The pups delivered were at term. What effect the injection had is a question.

SERIES VI. THE EFFECT OF FEEDING ON THE AMINO ACID CONTENT AND THE FERMENT STRENGTH OF THE BLOOD SERUM USING PLACENTA AS SUBSTRATE

It was thought valuable to observe the effect of feeding upon the amino acid content and the ferment strength of the serum in animals under the ordinary laboratory treatment. We did not desire to determine such on animals under constant diet, for such a procedure would have little bearing upon conditions ordinarily met with while using the laboratory animal. Ross (12) in this laboratory has reported carefully conducted experiments on the increase in amino acids one-half hour and four hours after feeding dogs one pound of ground lean meat. After one-half hour he finds no changes but four hours after feeding the amino acids are quite considerably increased. This increase is much greater than the increase observed on the less protein diet of the ordinary laboratory animal.

TABLE 5

The effect of feeding on the amino acid content and the ferment strength of the blood serum using placenta as substrate

EXPERIMENT	INTERVAL AFTER FEEDING	SERUM	SERUM	SERUM AND PLACENTA	SERUM AND PLACENTA
	min.	*before*	*after*	*before*	*after*
1	30	0.054	0.091	0.131	0.123
2	30	0.075	0.075	0.112	0.118
3	120	0.130	0.138	0.191	0.171
4	150	0.124	0.124	0.149	0.149
5	165	0.099	0.104	0.115	0.115
6	240	0.108	0.102	0.148	0.148
7	300	0.076	0.094	0.124	0.124
Average.....	.....	0.094	0.104	0.138	0.135
		Due to feeding 0.010			
		Due to ferment action 0.044			

Here we note a slight increase in the general average of amino acids when animals are fed upon the ordinary mixed food of the laboratory. On the other hand there is practically no change in the enzymes. It is possible that the latter is due to the inhibition of ferments by the increase in the lipoidal content of the serum.

CONCLUSIONS

1. Normal blood serum contains a weak proteolytic ferment which carries digestion to the amino acid stage. The concentration of this ferment is not constant.

2. There is an increased proteolytic ferment action of serum during pregnancy as shown by the dialysis method of Abderhalden. This cannot be detected by the Van Slyke method.

3. The injection of placental suspension does not give rise to an increased production or mobilization of ferments when tested by the dialysis method or the Van Slyke method.

4. The injection of the split products of pepsin-trypsin digestion of human placenta gives rise to an increase in the ferments. This increase can be shown by the dialysis method but not by means of the Van Slyke method.

5. The theory underlying the Abderhalden reaction as advanced by Abderhalden does not explain the increase in ferments found during

pregnancy. The basis of the theory would seem to be the entrance of split products of placental hydrolysis rather than of chorionic villi and cells.

6. The Van Slyke method is not applicable to determinations of the above character in which a non-soluble, moist, complex, protein substance is incubated with serum in a test-tube but without dialysis.

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THE INFLUENCE OF PARTIAL THYROIDECTOMY IN PIGS

C. C. PALMER

From the Veterinary Research Laboratories, University Farm, St. Paul, Minnesota

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INTRODUCTION

The work reported in this paper was undertaken at the suggestion of Dr. A. J. Carlson, with the view of determining if cretinism can be produced in this animal.

It is difficult to carry out complete thyroidectomy in the pig, owing to the fact that considerable accessory thyroid tissue is scattered along the entire cervical region, and is present in the thoracic cavity of some individuals.

Although there is a vast amount of literature available concerning thyroidectomy in experimental animals, that pertaining to the pig is very meager.

Horsley (1) in 1886, reported that removal of the thyroid in two pigs was followed by symptoms of tremor, leucocytosis and anemia with subnormal temperature. In 1892 (2) he reported that experiments by himself and Munk yielded negative results, but that the animals were not observed for a sufficient length of time. He leaves the impression that this group of pigs would have very likely developed the characteristic symptoms and died had they been observed longer. He also states that the results are much more serious in young animals, and that compensatory hypertrophy takes place very readily in young animals. He classifies the pig (with the sheep, goat and donkey) in the group of animals which slowly develop general cachexia, with death after thyroidectomy.

Eiselberg (3) removed the thyroid from a pig four weeks of age. He reports a marked retardation in growth, unaccompanied, however, by apathetic idiocy, the animal being as lively as normal animals.

Moussu (4) operated upon one pig fifteen days of age. It remained normal until about one month later when it began to show symptoms of myxedema. It became sickly in appearance, the skin became bald

in places, and in others was covered with remarkably long and thick bristles. There was swelling of the abdomen and formation of infiltrated cutaneous folds. There was also retarded growth. The animal died at the age of two months and four days. Post-mortem symptoms were stated to be similar to those found in human cretins.

The thyroidectomized and control pigs. From three pigs the thyroid was removed by the operation described below. Three other pigs were kept as controls. The six pigs were from the same litter of seven. The mother, a grade white sow was immune to cholera, and on April 15, 1915, gave birth to seven healthy strong pigs. In the litter were two females and five males. One female and two males were operated upon, and one female and two males were used as checks. The operated pigs consisted of a black and white male, a white male and a white female. The checks consisted of a black and white female and two white males.

The pigs, with their mother, were kept in a warm, clean box stall, and on bright sunshiny days enjoyed the freedom of an outside pen.

When the pigs were eight weeks of age, they were weaned, and then placed in pens along with the university herd. At first, the operated pigs were placed in one pen and the checks in another, and later placed all together in a large pen with an open yard adjoining. They were well cared for and given all they would eat of a properly balanced ration.

When four months of age the pigs were given a protective dose of hog cholera serum. Hog cholera existed on the farm and the pigs were vaccinated as a matter of precaution.

In November the pigs were placed in one of the hog houses of the serum plant. They were placed in a large inside pen and were well fed and taken care of. They were in a building which was used for quartering hog cholera virus pigs, and accordingly were carefully watched for any symptoms of cholera, but as none developed they were thought to be immune from the disease.

Anatomy of the thyroid. In the pig the thyroid consists of two lobes, closely attached to each other by means of connective tissue, so that an isthmus cannot be distinguished. The gland varies slightly in position in different individuals, but is always situated on the ventral surface of the trachea, and usually a couple of inches below the larynx, but may be in contact with it. Sisson (5) states that in the adult the lateral lobes are irregularly triangular in outline and in a large adult are two inches or more in length; and that the chief artery enters at the posterior extremity.

In the week old pig the gland is similar in general arrangement to that of the adult. Each lobe is thin, rather ovoid in shape, and about the size of a bean. The two lobes are intimately connected, except at the posterior end where each lobe is pointed. The gland varies in size, but on an average weighs about 0.5 gram. On section the alveoli are about twice the size of those in an adult normal hog.

The parathyroids in this animal, according to Ellenberger (6) differ from those found in other animals in that the median pair are missing. He further states that the lateral parathyroids in ruminants and pigs do not extend to the thyroid, but remain permanently at the cranial end of the thymus; and that in mature animals as the thymus disappears they are surrounded by a small amount of thymus tissue or fat. If the position of the lateral parathyroids can be in any way judged by the position of the thymus, they are quite apt to vary in position. Sisson (5) states that the thymus in the pig may extend up the cervical region to a varying extent, that it may extend to the larynx or even to the mandibular space. We have found the position of the thymus in the fetus to vary, but it is usually found at or near the thoracic inlet. Owing to the location of the parathyroids in this animal there is not much danger of removing them in a thyroidectomy; and the symptoms following such an operation should not be accompanied by the symptoms following a parathyroidectomy.

The presence of an accessory thyroid, somewhat oval in shape and about the size of a marble is quite constant at the thoracic inlet.

The operation. The three pigs were thyroidectomized aseptically and under ether anesthesia when eight, twelve and fifteen days of age, respectively. The thyroid lobes were found to be but loosely attached to the trachea, so that they were removed intact. The recurrent laryngeal nerves are not in contact with the thyroid, so there is no danger from this source in the pig. The operated pigs when returned to the mother, began to nurse immediately and in no case did they miss a feed. The young pig takes the ether quite readily, only a few inhalations being required to produce anesthesia. The wounds healed by primary intention in all cases and the sutures were removed on the seventh day.

Results. During the first two months no changes in physical condition could be observed. The operated and control pigs were of equal size and weight.

The first pig to show any change was the operated white sow. She developed many circumscribed, inflamed, swollen areas about the size

of a dime, along the ventral surface of the abdomen, which were very resistant to treatment. The only other pig to show such lesions was the operated white boar. No specific cause for this condition could be determined, and in pigs, skin lesions of this kind are unknown to the writer. Scrapings from the diseased areas were negative when examined under the microscope. Owing to the slop feed which the mother received and the nature of the animals, the cement floor of the pen in which these animals were kept was wet most of the time, but plenty of clean dry straw was provided, and in general the pen was much cleaner and dryer than the average pig pen. This skin disease could hardly be attributed to the pen because only two of the pigs showed symptoms (both operated pigs) and such lesions are not common in pigs which are quartered in much poorer pens.

An outbreak of necrobacillosis existed among the young pigs of the university herd, but in these the lesions were confined to the mouth and internal organs. These sores on the operated animals did not become necrotic, nor could the necrosis bacillus be demonstrated. After several weeks the lesions disappeared, the affected animals being given plenty of sunshine and antiseptic, astringent baths.

The next indication of a lowered resistance was manifested by the operated black and white boar. In June he was noticed to be lame and upon examination, one of the digits of the left fore limb was found to be swollen and painful. No infection developed, the injury was probably a bruising of the tissues, which was very slow to heal. The animal remained lame for over six weeks. This condition is rarely seen in the pig and when bruising does occur the lameness is of short duration unless infection develops.

During the early summer when about three months old, all of the pigs showed symptoms of parasitism, especially lung worms, with much coughing. No treatment was given but this invasion interfered with the growth and thrift of all the pigs. The symptoms were more marked in the operated pigs and although the rate of gain was about equal in the two groups, the operated pigs made a much slower recovery than the normal. The operated white boar seemed to suffer the most, and became the smallest pig in the litter. His hair coat was rough, and the bristles came out. He became almost entirely bald over his body and the skin very red and congested. He developed a "pot-belly" and a myxedematous appearance. He was not expected to live.

In the fall when the cold weather set in, the pigs began to improve and gain in weight more rapidly and none of them showed any signs of

parasitism. Even the white boar which was not expected to live showed a very marked improvement, although a little undersized. The pot-belly disappeared, the hair coat improved and other than being a little smaller he could not be differentiated from the others. The parasites did not interfere with the growth and development of the testicles in the male pigs, and it became necessary to castrate them. They were castrated on October 13, 1915, with the exception of the black and white thyroidectomized boar which was left entire, because it was intended to experiment with his fertility. No differences were noted in the size and appearance of the testicles of the operated and check pigs. The testicles represented 0.76 per cent and 0.56 per cent of the body weight in the check pigs and 0.77 per cent of the body weight of the operated pig.

The body temperatures of all the pigs were taken at frequent intervals, but they showed no variations from the normal.

During the winter the operated pigs grew well but did not gain in weight as rapidly as the check pigs. The average weight curve is shown in the following chart. It will be noticed that during the first few weeks the weights of both groups were equal; from the fourth to the ninth week there was quite a marked difference in the rate of growth in the two groups, but after the ninth week the rate of growth in the operated pigs was equal to that of the checks. This equal rate continued until the twenty-seventh week, when the rate of growth increased in the checks, but remained about the same in the operated pigs. At forty weeks, which is about the time of market maturity in this animal, there was an average difference of 30 pounds between the checks and operated pigs.

Sexually, all of the males were very active, but owing to the fact they were grade stock there was no opportunity to try out the reproductive powers of the thyroidectomized boar on mature breeding sows. Early in November the two sows (operated and check) came in heat. They were bred to the operated boar, and conceived, as dioestrum did not recur. Post-mortem on the two sows was held shortly before the time of parturition. The operated sow presented one *mummified fetus* in the uterus, and the check sow presented one *fully formed fetus*, which appeared normal and probably would have been born in a short time. This fact is significant, as normal sows bred to normal boars usually have five or six pigs at the first litter, and older sows may have as many as ten to fifteen. That the operated boar retained his sexual activity was demonstrated by the fact that whenever any of the sows

in his building were in heat he made violent efforts to reach them and several times did get over the high partition of his pen.

Post-mortem findings.[1] On February 4, 1916, the operated white sow was reported sick by the feeder, and when seen was found to be in a dying condition. The feeder reported that for a couple of days she had not been eating very well. Post-mortem revealed pneumonia and

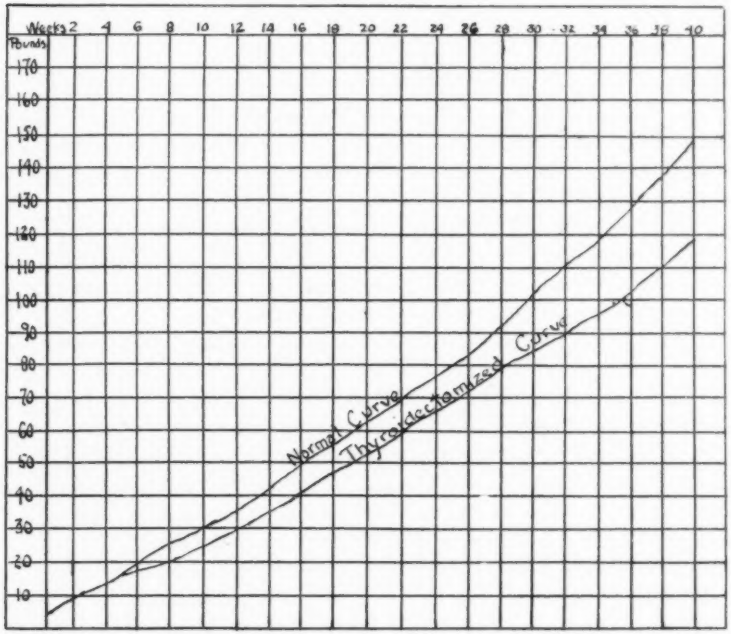

Fig. 1. The growth curves of three normal and three thyroidectomized pigs

pleurisy involving both lungs and the parietal and visceral pleura of both sides. The digestive organs showed a gastritis. Abdominal and thoracic lymph glands were congested. All other organs were normal, except the uterus; here there was considerable exudate and a mummified fetus was found. The head of the fetus was fairly well developed,

[1] Dr. Kernkamp of the serum laboratories coöperated in the post-mortem examinations.

the body small in diameter, but very long, and the legs were not developed to any appreciable extent. The region of the larynx and trachea of this operated animal was carefully examined and numerous gland-like structures varying in size from a small seed to a large bean were found and retained for microscopic study.

The pigs were carefully watched and none of the remaining animals showed any signs of sickness, but on February 14, 1916, two more were found dead. The pigs had been seen last by myself the day before and at that time they all appeared normal. The dead pigs were the operated white male and the check, black and white sow. The check sow was posted first, and the only lesions found were in the lungs. These organs showed lesions of pneumonia involving only the distal parts of the cardiac lobes. The uterus was normal and contained one normal fetus. The operated white male showed very marked lesions in many organs. There was extensive pneumonia and pleurisy. Digestive tract showed a congested intestine. The spleen was hemorrhagic and the kidneys showed a few petechiae. The lymph glands of the entire body were swollen and congested. All gland-like structures in the cervical region were removed and sectioned.

Although the lung lesions in the thyroidectomized animal were the predominating post-mortem findings, and the primary cause of death was very likely a pneumonia, the case resembled hog cholera somewhat. Therefore, when the remaining operated pig, the black and white boar, showed a slight temperature and was off feed a few days later, he was given 60 cc. of hog cholera serum. A blood inoculation would have been made from this animal to determine if he really had cholera, but no known susceptible pigs could be obtained at that time. The boar's condition remained about the same for a few days, then he grew worse, and died on February 20, 1916. Post-mortem examination revealed pneumonia and pleurisy involving both lungs and pleura, gastritis with blood stained stomach contents, lymph glands of thorax congested and swollen; kidneys showed a few petechiae, but all other organs normal. All glands from the cervical region were removed and sectioned as in the case of the other operated pigs.

The remaining check pigs did not become sick, the appetite remained good and they did not show any rise in body temperature.

All of the thyroidectomized pigs died, the female with the mummified fetus first, and the boar last. They all showed extensive lesions with those of pneumonia and pleurisy predominating and constant. Only one check pig died. Death in all these animals was, in the opinion of

Dr. Kernkamp and myself, caused primarily by pneumonia, which is rather common in pigs during the winter months. During cold weather the pigs will pile up on one another. Those on the bottom become very warm and later when they come in contact with the cold air, chill, and if their resistance is low are quite apt to develop pneumonia.

While some of these pigs showed slight lesions of cholera, they were not typical. Further, these pigs had been given serum when young and very likely became actively immune. If they were not, they would certainly have shown symptoms in the fall shortly after being placed in the shed occupied by virus pigs. The boar was given the serum when he first showed signs of sickness. Serum is sometimes effective as a curative agent for cholera, especially when administered early and in large doses.

At the time of the post-mortem examination all glands in the region of the larynx and trachea were removed and sectioned. These structures varied in size from a small seed up to that of a large bean or hickory nut. Most of the small glands proved to be lymph glands, but in each operated pig several glands were found which proved to be accessory thyroids. The glands were for the most part located at or near the thoracic inlet along the trachea. The largest accessory thyroid was found in the case of the black and white boar. The gland was 4 cm. long and 2.5 cm. wide and 0.5 cm. thick, located along the ventral surface of the trachea at the thoracic inlet. The alveoli in the accessory thyroids were about the size of those found in the thyroid of the week old pig.

Discussion of the results. Definite conclusions cannot be drawn from such a small number of animals, but owing to the fact that the variations from the normal in the three operated pigs were quite constant, we believe the following comments are warranted.

Our results are not in accord with those obtained by Horsley, Munk and Moussu, but are in general the same as those reported by Eiselberg.

It is difficult to account for the results obtained in Moussu's one case. One of our animals (the white male) when about three months of age began to show symptoms quite similar to those described by Moussu, and if the animal had died at this time we would have believed that this pig was developing into a cretin, but died before the syndrome was complete. This pig, however, soon began to improve and when fall came, looked and acted similar to the other pigs, though slightly undersized.

Moussu does not describe the method of feeding and quartering his

animal, but it is well known that pigs and especially young pigs, if not fed properly and quartered in sanitary pens are quite likely to develop a pot bellied appearance, an unthrifty hair coat and general cachexia. Such a condition is usually the result of an invasion of parasites.

Another possible explanation of Moussu's results is, that the particular animal upon which he operated had a very small amount of accessory thyroid tissue. (In posting pigs I have sometimes found the large accessory thyroid, usually located at the thoracic inlet, to be absent.)

Horsley's pigs developed a condition of general cachexia and died after a long period of this depression. True cretinism would very likely have started to appear when the animals were much younger. Death in these cases was very likely the result of a lowered body resistance.

Although the details of Eiselberg's work are not available, our results are, in general, in accord with those obtained by him. Our operated pigs were somewhat undersized. This change was more marked during the last two months of their lives, and would have very likely increased if the pigs had lived longer. It is probable that this decreased rate of growth was the result of the lowered body resistance, or may have been due to disturbances in metabolism as a result of the diminished quantity of thyroid tissue.

All of the thyroidectomized pigs showed a marked lowered body resistance at some time during their lives. This fact is demonstrated by the skin disturbances, the parasitic invasion and finally the deaths from pneumonia. On post-mortem there were extensive tissue changes in the operated pigs which died from pneumonia, but only slight lung lesions existed in the one check pig which died from this disease.

Before the fatal pneumonia, a stranger had difficulty in distinguishing the operated from the normal pigs. Other than the slight differences in weight, there were no distinguishing clinical features.

The apparent effect upon the reproductive powers is interesting, but further work along this line is needed before any statements can be made.

SUMMARY

1. Extirpation of the main thyroid gland in young pigs does not induce cretinism, at least within a period of nearly one year. There is a slight retardation in growth, but the operated pigs act similar to the controls, except that they show a marked lowered resistance to infection.

2. Post-mortem examination of the thyroidectomized pigs revealed a marked hypertrophy of the accessory thyroid tissue.

3. The main thyroid gland plays an important part in the normal body resistance to infection and when this structure is removed the accessory thyroids cannot completely compensate.

4. In pigs a degree of hypothyroidism, not sufficient to lead to marked changes in physical appearance of the animal, lowers the resistance to infection to quite a degree, and impairs the functions of reproduction.

I wish to express my appreciation to Dr. Carlson for his valuable criticisms, and to Drs. E. P. Lyon, F. H. Scott and C. M. Jackson for their valuable suggestions.

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PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY

TWENTY-NINTH ANNUAL MEETING

New York City, December 27, 28, 29, 30, 1916

IN MEMORIAM

Isaac Ott, A.M., M.D., by Albert P. Brubaker.
Joseph Hoeing Kastle, Ph.D., by A. S. Loevenhart.
Allen M. Cleghorn, M.D., C.M., by Shepherd Ivory Franz.
Thomas Gregor Brodie, M.D., F.R.S., by A. B. Macallum.

The diastatic activity of the blood in diabetes. VICTOR C. MYERS and JOHN A. KILLIAN.

Although it has been recognized since the time of Magendie that blood possesses active diastatic properties, no studies of a conclusive nature appear to have been published relative to the activity of the blood diastase in human subjects suffering from diabetes. This has probably been due to the lack of a sufficiently delicate and accurate method.

It has been found that the procedure introduced by Lewis and Benedict[1] for the estimation of the sugar of the blood, may be excellently utilized in the estimation of its diastatic activity. Two 2 cc. samples of oxalated blood are taken, one being employed as a control. The control tube is made up to 10 cc. with distilled water and the tube to be employed for the test to 9 cc. Both tubes are now placed in a water bath at 40°C. As soon as the contents of the tubes have been brought to this temperature, 1 cc. of 1 per cent soluble starch is added to the second tube, the tube mixed, and incubation then carried out for exactly fifteen minutes at 40°C.

After the incubation has been completed, about 1 gram of dry picric acid is at once added to each tube and the mixtures stirred. When the proteins are precipitated, the tubes are centrifuged and the supernatant fluid filtered. The sugar in 3 cc. portions of the filtrates is now estimated according to the technique described by Myers and Bailey.[2] Correction is made for the sugar originally present in the blood (with the aid of the control) and for the slight reducing action of the soluble starch. The results have been recorded in terms of the percentage of

[1] Lewis and Benedict: Journ. Biol. Chem., 1915, xx, 61.
[2] Myers and Bailey: Journ. Biol. Chem., 1916, xxiv, 147.

the soluble starch (10 mgm.) transformed to reducing sugars (calculated as glucose) by the 2 cc. of blood employed.

It is believed that 10 mgm. of starch furnish a sufficient substrate for all ordinary conditions with human blood (except possibly in very severe diabetes), since practically identical results have been obtained with amounts of starch up to 40 mgm. Glycogen offers no advantage over soluble starch, since the results are essentially identical. The possible error of glycolysis would appear to be a negligible one during a fifteen minute period of incubation.

The diastatic activity of the blood, with this method, has been found to vary from 15 to 25 in a variety of miscellaneous conditions in the human subject. In cases of advanced nephritis with nitrogen retention and hyperglycemia, figures from 30 to 40 have been observed, while in eleven cases of diabetes the values ranged from 40 to 74. These results would suggest that the increased diastatic activity in both nephritis and diabetes (as shown by the analyses of the blood) may be the important factor in the production of the hyperglycemia in these conditions.

Observations concerning fat feeding. F. M. ALLEN.

The observed effects of fat feeding are classifiable in four divisions.

1. Effect on acidosis. It is well known that the ordinary laboratory animals show no ketonuria on fasting. There is no distinction between carnivora and herbivora in this respect. The experiments confirm the statement of Neumann and others, that young puppies show fasting ketonuria. Their weakness is the main difficulty for experimental uses. Adult dogs by partial pancreatectomy become subject to acidosis under three conditions:

A. Acidosis on feeding. Suitable fat diet gives rise to acidosis in severely diabetic dogs possessing sufficient power of fat digestion. Breakdown of digestion is the principal difficulty to be avoided. With successful management, the acidosis proceeds to typical diabetic coma. The fat ration employed has generally been 100 to 250 grams of suet daily. As in human diabetes, protein and carbohydrate fail to stop this acidosis, so that dogs go into coma on full mixed diet. The carbon dioxide capacity of the blood plasma falls only in the more severe later stage, but in coma approximates that of human patients. Doses of alkali easily maintain the blood alkalinity but do not perceptibly lengthen life. The excretion of acetone bodies is generally much less than in human patients, but the concentration in the blood is fully comparable.

B. Acidosis on fasting. When a dog with potentially severe diabetes is kept free from glycosuria and gradually fattened by means of a low protein, high fat diet, glycosuria ultimately develops and a condition can be attained in which the animal develops fatal acidosis on fasting, reproducing the condition and the symptoms of certain human patients.

C. Acidosis without glycosuria. Partial pancreatectomy creates susceptibility to acidosis even when glycosuria is entirely prevented by

diet. Such animals on suitable fat diets develop ketonuria far more readily than normal dogs.

2. Effect on glycosuria. Fat feeding alone never gives rise to glycosuria, but in predisposed animals and patients free from glycosuria on a given diet, the addition of fat results in glycosuria.

3. Toxicity in normal animals. Young puppies are easily killed by excessive fat diet, especially by feeding pure fat after fasting for a few days has produced ketonuria. Adult dogs can be made to develop both acetonuria and intoxication if the fat feeding is pushed long enough. There is no parallelism between acetone bodies and intoxication in these animals. The symptoms are loss of hair, eczema, loss of weight, and very pronounced terminal ataxia and generally convulsions.

4. Lipemia. Partial pancreatectomy occasions only a slight lipemic tendency in absence of glycosuria, and glycosuria produced by phloridzin is known to be accompanied by only slight lipemia, but when the partially depancreatized animals are allowed to develop severe glycosuria, they become susceptible to true diabetic lipemia on fat feeding. As high as 10 per cent fat has been found in their plasma. The finer characteristics and the reasons for variations between individual animals are under study.

The experiments indicate that acidosis is not due solely to lack of carbohydrate, but that the pancreatic function is somehow concerned, possibly only indirectly through the liver or otherwise. A wide range of experimental material is afforded for researches not limited to diabetes, dealing with endocrine functions, enzymes such as lipases, lipoid metabolism from the standpoint of both chemistry and morphology, etc.

The cause of the increased heat production following pancreatectomy in the dog. JOHN R. MURLIN.

Three experiments were reported in which the heat production by the indirect method was determined before and after pancreatectomy; and, immediately following each respiration experiment, blood was drawn and examined for blood fat. In one experiment, also, the CO_2-carrying power of the whole blood was determined by the Haldane method. Thus it was possible to compare the heat production with the intensity of the protein metabolism (N output in urine) with the concentration of blood fat and with the actual acidosis.

Two of the dogs were fed both before and after pancreatectomy, one fasted throughout. In the fed dogs the heat production rose on the second day to 31 and 42 per cent, respectively, above the normal metabolism. In the fasting dog it rose to only 7 per cent above the normal. In the last-named dog the increased protein metabolism alone was sufficient to more than account for the heat production; but in the dogs which were fed it was not sufficient.

When fat is injected directly into the circulation[1] or when fat alone is fed[2] the increased heat production due to the dynamic action of fat runs

[1] Murlin and Riche: Proc. Soc. of Exper. Biol. and Med., 1915, xiii, 7.
[2] Murlin and Lusk: Journ. Biol. Chem., 1915, xxii, 15.

parallel to the concentration of blood fat. But after pancreatectomy it does not. In none of the three cases did the maximum heat production coincide with the maximum concentration of blood fat. Since the carbon dioxid carrying power of the blood rose after pancreatectomy in the only case studied for this factor, the increased heat production can not be said to depend upon the acidosis. Furthermore, the exact course of the heat production, which was perfectly parallel for the two fed animals, could not be accounted for by any combination of the protein and fat factors. The subject is being investigated further.

The use of cotton seed as food. THOMAS B. OSBORNE and LAFAYETTE B. MENDEL.

Both the *globulin* and the *total alkali-soluble protein* isolated from cotton seed as well as cotton seed *meal* and *flour* respectively are adequate sources of protein in the ration of growing rats. Small supplements of the meal or flour make the proteins of less efficient feeds such as "corn gluten," distillers' grains, etc., suitable for growth.

Cotton seed *meal* and *flour* contain "water-soluble" and possibly some of the "fat-soluble" vitamines.

Feeding with untreated cotton seed *kernels* leads to nutritive failure. They apparently contain something which is either toxic in itself, or renders the food so unpalatable that rats refuse to eat it. This results in so-called cotton seed injury. When the *kernels* are thoroughly extracted with ether or suitably steamed, rations containing them can be used with success. Food containing small quantities of the *ether extract* becomes unsuitable for nutrition.

Both the crude cotton seed *oil* prepared from heated kernels and that pressed from cold kernels can be fed without detriment. The cotton seed *residue* from the *cold pressed* oil is no better than the untreated kernels for nutrition.

Some of these facts corroborate observations by Richardson and Green and by Withers and Carruth.

The influence of certain conditions on the rate at which epinephrin is liberated from the adrenals into the blood. G. N. STEWART and J. M. ROGOFF.

1. Using rabbit intestine and uterus segment method for assaying epinephrin in adrenal vein blood, we have been unable to demonstrate any decided increase in the rate of liberation by stimulation of sensory nerves or asphyxia. This agrees with our previous results[1] by another method (collection of adrenal blood in a cava pocket and determination of its effect on blood pressure and pupil of the same animal when the pocket is released).

2. The concentration of epinephrin in the blood of the adrenal veins tends in general to increase as the rate of flow through the adrenals diminishes, the quantity liberated being proportional to the time rather than to blood flow.

3. For this, and possibly for other reasons the concentration of epi-

[1] Stewart and Rogoff: Journ. Pharm. and Exper. Therap., 1916, viii, 479.

nephrin in adrenal blood tends to be greater in later than in earlier samples.

4. It follows that a change in the rate of liberation of epinephrin can not be deduced from a change in the concentration (as determined by segments or strips) unless the rate of flow of the adrenal blood has been measured.

5. Cava blood withdrawn by a catheter whose orifice is central to the adrenal veins does not in general give reactions with rabbit intestine and uterus segments which would permit the detection of epinephrin, even when the concentration in the adrenal vein blood is proved to be capable of giving marked reactions. The reason is the dilution of the adrenal vein contribution.

6. Even when serum is used instead of blood the results (for catheter specimens) have been negative. Yet when adrenalin is added to blood the separated serum contains practically all the adrenalin (by colorimetric, rabbit segment and blood pressure tests on the pithed cat).

7. Our attempts by the catheter method to demonstrate an increase in the rate of liberation of epinephrin by sensory stimulation, asphyxia and emotional disturbance have not been successful.

8. When the blood flow in the cava is sufficiently slowed the concentration of epinephrin in catheter specimens may be detectable with rabbit segments, although falling below the threshold of detection in earlier specimens when the cava flow was larger. This would not prove that the rate of liberation of epinephrin had been increased by any procedure concomitant with the slowing of the blood flow. For if the rate of liberation remained the same, the concentration in the catheter blood would necessarily increase as the flow in the cava lessened.

The blood lipoids in nephritis. W. R. Bloor.

Lipemia has occasionally been reported in nephritis and because of its importance in the study of fat metabolism an examination of the blood lipoids in a series of cases of severe nephritis was undertaken. The results showed mainly marked increases in the total fatty acids (due to fat) in the blood plasma and of lecithin in the corpuscles, with less marked and less frequent increases of fat and of cholesterol in the corpuscles. These abnormalities are essentially those found in the blood in alimentary lipemia and their presence in nephritis is believed to be due to a retarded fat assimilation which is regarded as one manifestation of a general phenomenon produced by the acidosis present in this condition.

Concerning the lipoids of the blood in renal conditions, with special reference to the cholesterol content. Albert A. Epstein and Marcus A. Rothschild.

In the interpretation of phenomena associated with renal conditions, the changes found in the blood and urine are ordinarily attributed to the pathological process and disordered function of the kidneys. By a

comprehensive study of the chemistry of the blood effusions and of the urine in renal and other conditions, the conclusion has been reached that a type of renal affection exists, commonly known as chronic nephrosis, in which the pathological and chemical basis is that of a nutritional disorder. One of the authors (Epstein) has on several previous occasions pointed out the fact that amongst the cases of chronic parenchymatous nephritis there is a group which is essentially different from all other renal affections, and is fundamentally a constitutional disturbance.

This conclusion is based on the profound changes, particularly in the protein content of the blood serum, encountered in this type of nephritis, and also from the character of the chemical composition of the effusions as compared to that observed in other conditions.

By means of the newly developed methods for the examination of fats and lipoids in the blood, it has been possible for us to examine nearly one hundred cases of renal disease and allied conditions. The results may be briefly summarized as follows: In chronic nephrosis of the type referred to, particularly in an edematous stage, the lipoids of the blood are exceedingly high; the cholesterol content, to which special attention has been given in this study, has been observed as high as 1.226 grams per 100 cc. In other renal conditions, including surgical cases, no such increase has been encountered. Occasionally a moderate rise has been found, but as a rule this was associated with some other superimposed condition.

In uremic cases, particularly those showing high nitrogen retention in the blood, the lipoid content is very much diminished. The cholesterol in such cases may fall as low as 0.080 gram per 100 cc. Several effusion fluids from cases of chronic nephrosis have been examined and only traces of lipoid have been found in them. The urines of such cases, when rich in protein material, show the presence of small amounts of lipoid.

The origin of the increase of the lipoid content of the blood in the parenchymatous form of nephritis is not quite clear. Undoubtedly a large portion is due to ingested lipoid material. A certain part may be ascribed to mobilization of body fat, particularly as such cases when recuperating show extreme emaciation. This high-grade form of malnutrition is undoubtedly associated with considerable tissue degeneration, so that much of the lipoid in the blood must be attributed to that source. At any rate, the finding evidently represents a condition of fat non-utilization comparable to that found in diabetes and other nutritional disorders. This is evidenced also by the fact that when a diet consisting of large amounts of protein, a small amount of carbohydrate, and practically no fat, is administered for a long period of time, the excessive lipemia and lipoidemia gradually subside.

Observations on acid base equilibrium in the body. W. McKim Marriott and John Howland.

Known amounts of hydrochloric acid, of acid sodium phosphate and of sodium phosphate of blood reaction were administered to normal

adults. Determinations on the urine showed that hydrochloric acid administration led to an increase in titrable acid (A) and a proportionate increase in ammonia excretion, the ratio A/NH_3 remaining constant. Acid sodium phosphate increased the titrable acid excretion but had no effect on ammonia excretion. The ratio, A/NH_3 was greatly increased. Administration of phosphate mixtures of blood reaction ($p_H = 7.4$) led to a slight increase in the titrable acid and to a distinct diminution in ammonia excretion. The A/NH_3 ratio was considerably above normal limits.

These findings may be taken to explain, in part, at least, the low excretion of ammonia in the acidosis of nephritis, it having been previously demonstrated that this condition is accompanied by a retention of acid phosphate.

The movements of the artery within the compression chamber during indirect estimations of the blood pressure. JOSEPH ERLANGER.

This report is based mainly upon records (made with the aid of a compression chamber applied to the dog's femoral artery) reproducing the movements of the walls of the artery within the compression chamber. The records made during steady decompression show that the pulse does not penetrate the full length of the compressed artery when it first enters it; rather the penetration depth increases at the rate of 1 cm. per pressure decrement of 2 mm. Hg. Once blood has entered the artery there is not time enough between pulses for the artery to completely empty itself. This residue of blood increases rapidly at first until a thickness of about 0.08 to 0.24 mm. is attained, then more slowly if at all, and again more rapidly and at an accelerating rate before the fourth sound-phase begins. This changes to a retarding rate of increase with the first of the fourth sound-phase pulses, because the artery at this time fails to collapse during diastole beyond its full but undistended size. During the whole of the first-to-fourth sound-phase the pulse, especially in the lowermost parts of compressed artery, shows a pre-anacrotic negative wave. This wave, as demonstrated by other methods, develops when a pulse passes through a localized compression constriction of a tube. It disappears when the tube is no longer constricted below its full but undistended size. The first sound-phase begins shortly after the pulse penetrates beyond the lowermost parts of the compressed artery; the fourth sound phase, at the instant during diastole the artery fails to pass below its stretched size. The behavior of the anacrotic limb of the pulse, as well as certain other phenomena, furnishes evidence in support of the water hammer theory of sound production. The anacrotic limb varies in such a way as to account for the sounds of the first, third and fourth phases. The elevation of the crest of the pulses increases rapidly at first, then more slowly, and again very rapidly as the fourth sound-phase is approached. The last rapid increase is mainly the result of the damming back of blood. The amplitude of oscillation at any point on the artery is given by the difference between the systolic and diastolic levels. The oscil-

lation curves have the usual form, but the sudden diminution in amplitude usually seen at diastolic compression is exhibited best, sometimes only, by the middle region of the compressed artery.

The veno-pressor mechanism. YANDELL HENDERSON.

By the veno-pressor mechanism I mean a third factor in the circulation which regulates the venous return to the right heart—the heart and the control over the arterioles by the vaso-motor nervous system being the other two. The following experiments throw some light on its nature. When the intestines are traumatized, either mechanically, or with mustard oil, the efferent vessels (mesenteric veins) contract and dam the blood back in the tissues, thus producing congestion and decreasing the return of the blood to the heart. This occurs equally well after section of the splanchnics. It is the essential feature of circulatory failure in experimental shock. The phenomenon is essentially the first stage of inflammation and is possibly related to the observations of Ninian Bruce on this matter. The same mechanism is affected by forced breathing in a normal man, as can be demonstrated when venous pressure is determined with the subject lying head down at an angle of 30°. The measurement of venous pressure in this position renders the estimation of this important function feasible to an extent otherwise unattainable, especially when as in sickness the venous return to the heart is diminished.

The effect of pneumothorax in the dog and cat. H. C. JACKSON and CHAS. J. IMPERATORI.

By pneumothorax in the dog and cat we mean a *double pneumothorax.* Single pneumothorax with the existing apparatus and our present methods is impossible to obtain. It would seem that some observers are of the opinion that there is a natural or anatomical opening between the two pleural cavities. Repeated dissections done on dogs but mostly on cats seem to show that there is no natural or anatomical communication. Test animals were bled to death and then immediately fixed in formalin solution.

Methods of inducing pneumothorax were, (a) by puncture of the chest wall, or (b) by puncture of the lung from within a bronchus, thus gaining entrance to the pleural cavity. Practically all the work was done by puncture through a bronchus.

Immediately following puncture the animal shows signs of embarrassed respiration and is dead in from two to four minutes from what might be termed a very rapid asphyxia.

Animals that were punctured from within the bronchus died more rapidly than those in which the chest wall was punctured. The normal negative pressure in both pleural cavities after puncture immediately becomes positive. There does not seem to be any appreciable interval of time between the change from negative to positive pressure on the side *not* punctured. Autopsy findings were collapse of *both* lungs regardless of which side was punctured.

Post mortem examinations all showed a collapsed condition of both lungs and a rupture of the pleural septum just behind the sternum, either a tearing of it or in the majority of instances a sort of peppering of this septum with small holes.

Simultaneous collapse of both lungs by the careful use of fluids, colored fluid being used on one side, resulted in the colored fluid remaining in its side and in death from asphyxia. But if the animal made violent efforts at respiration, a rupture of the septum invariably resulted. It might be of interest to note that those animals where the lung was punctured by the bronchoscope and air admitted to the pleural cavities, and who were rapidly becoming asphyxiated, could be resuscitated by puncturing the chest wall. By closing this opening in the chest wall they could again be asphyxiated; this procedure could be repeated many times, that is, alternately asphyxiating and resuscitating the animal.

Pneumothorax and the subsequent collapse produce, as it were, a clamping off of the pulmonary vessels and a rapid asphyxiation.

Effects of physical training on pulse and blood pressures during activity and during rest. PERCY M. DAWSON.

The subject of these studies is the writer. The periods of observation were two to three months long, one period being in each of the years '14, '15 and '16. During these periods blood pressure and pulse rate determinations were made from four to seven times a day. Only four series of these observations are included in this paper, namely, those made on rising, at noon, in the late afternoon, before retiring. The auscultatory method was used in blood pressure determinations, four to ten readings being taken on each occasion and the average being then computed. In '14 the subject frequently ran three miles; in '15 he performed gymnastic exercises; in '16 played tennis. It was therefore possible to study the effect of physical exercise on the resting pulse and blood pressure and also upon the cardio-vascular reaction to standard physical tests (cycle ergometer). The following are the more important results.

Resting pulse rate is decreased especially in '14 and '16, and the extent of the diurnal variation is decreased.

Resting blood pressure observations are most complete in '16. They show that both pressures are increased on rising and in the evening, both fall at noon but in the afternoon the rise in systolic pressure is accompanied by a fall in the diastolic. The diurnal variations are decreased but suffer no qualitative change.

Resting pulse pressure is increased in all cases.

Resting pp $\times$ pr is affected neither conspicuously nor constantly.

The effects of several weeks of systematic exercise upon the blood pressure reaction to physical tests (cycle riding) are these: (1) the systolic pressure rises much higher (cf. 170 and 206); (2) the diastolic after a short rise falls to or below normal before the completion of the test; (3) the pulse pressure is much greater (cf. 62 and 114); (4) the

pp × pr is greatly increased (cf. 6820 and 12540); (5) the after effects (negative phase and fall in diastolic pressure) are considerably reduced.

If the untrained subject performs two cycle tests with a day of rest between them, the first ride is seen to modify very greatly the cardiovascular reaction to the second ride. This has been studied only in the period immediately following the exercise. We then see a higher systolic, a lower diastolic, an increased pulse pressure (cf. 46 and 60) an increased pp × pr (cf. 5980 and 7800).

During the period of observation the subject caught "cold" twice, once before and once after the period of training. To the subject the two infections seemed to be identical in character, duration and severity. The only objective sign on both occasions was the increase in pulse rate. The maximum in the first case was 86 and in the second 69.

Some electrical phenomena of animal tissues. ROBERT GESELL.

Renal electrical variations were studied during sodium sulphate and "saline" diuresis. Though the details of the deflection varied the usual result was in each case increased negativity running roughly parallel with the secretion.

Certain exceptions—such as reversal of the electrical variation, the occurrence of electrical variations though no noticeable change in renal activity occurred, and the production of copious diuresis unaccompanied by marked electrical variations pointed out difficulties in the interpretation of secretion by means of the accompanying electrical changes. The problem temporarily resolved itself rather into the interpretation of the electrical changes than the interpretation of secretion by electrical changes.

Of the tissues studied, with the exception of the testicle, decreased blood supply produced a decreased negativity. In the case of the salivary glands, two results were obtained, following pilocarpin injection. If the salivary secretion was accompanied by increased negativity, decreased blood supply produced the usual decreased negativity. If the secretion was accompanied by a reversal of the usual electrical variation, decreased blood supply produced increased negativity.

The data from the use of adrenin, pilocarpin and direct stimulation of nerves as yet are not sufficient for reaching a generalization concerning the electrical effect of sympathetic and autonomic stimulation.

The electrical variation of the submaxillary gland of the dog produced by faradic stimulation of the chorda tympani showed four components which to a certain extent were under experimental control. The first component was produced by a short weak stimulation. The second component was added by a stronger stimulation and the third by a still stronger or longer stimulation. The fourth component seems to vary with the intensity of stimulation.

Obstruction of the salivary duct just prior to stimulation of the chorda tympani changes the contour of the deflection.

If all the components of the electrical variation produced by stimu-

lation of the chorda tympani were present during secretion produced by the injection of pilocarpin they were fused into a smoother curve.

Obstruction of the salivary duct during secretion following injection of pilocarpin produced a marked deflection, so did release of the obstruction.

Injection of atropin after effective pilocarpin injection produced a sudden decrease of the original deflection with stoppage of secretion.

Stimulation of the chorda tympani after injection of atropin sufficient to stop or prevent a noticeable secretion still produced a small electrical variation. A method was devised for recording electrical variations on smoked paper.

Experiments on the relation of blood pressure to urine formation. A. N. Richards and O. H. Plant.

By a method previously described[1] rabbits' kidneys were perfused with hirudinized blood under conditions which not only permitted the regulation and measurement of blood flow through the organ, but also prevented alterations in blood flow as a result of changes in resistance in the renal circulation. Keeping blood flow constant, increase of blood pressure in the renal circulation by (1) partial obstruction of the renal vein; (2) stimulation of the splanchnic nerve; or (3) injection of adrenalin was found to increase urine elimination.

Evidence was presented which indicates that adrenalin causes constriction of the efferent vessel of the glomerulus and therefore, under the conditions of these experiments, causes increase in pressure in the glomerular capillaries. For the volume of the kidney, perfused at a constant rate, may show no change or may undergo increase after the injection of amounts of adrenalin which cause marked constriction of its vessels as shown by rise of pressure in the renal artery; whereas the forelimb or a loop of intestine, perfused under identical conditions, invariably decreased in volume after any dose of adrenalin which caused constriction of its vessels. This fact is taken to mean that a passive distension of the glomeruli results from constriction of the vasa efferentia which is great enough to prevent shrinkage of the kidney.

The fate of phenolsulfonephthalein when injected into the animal organism; factors other than the kidney influencing its retention. E. C. Kendall.

Phenolsulfonephthalein may be destroyed within the animal organism by being reduced. When once reduced, it is not possible to reoxidize the substance within the body to its original color. Blood fully oxygenated and reduced will not destroy phenolsulfonephthalein. Therefore, the destruction is caused by a reducing substance within the tissues and not within the blood. The oxygen of the blood is sufficient to fully meet the demands of the reducing action of the tissues, and will prevent a destruction of the dye. As high as 98 per cent may be recovered during a continuous injection of the substance provided glucose is the substance being metabolized. When amino acids and fats are

[1] Journ. Pharm. and Exper. Therap., 1915, vii, 488.

being metabolized, the amount recovered may be much less. Fundamentally, the phthalein test measures the relation between the supply of oxygen of the blood and the reducing power of the tissues.

Factors other than the kidney which influence its so-called retention are poor oxygen-carrying capacity of the blood, sluggish circulation, improper aeration by the lungs, and any other factor which produces asphyxia or a condition of acidosis.

The action of diuretics on the denervated kidney. WILLIAM C. QUINBY.

That the plexus of nerves going to the kidney has a marked vasomotor function is everywhere admitted. Besides this action, however, certain observers have claimed that some of these nerves were of specific secretory function. By others this has been denied. Certain investigators attempt to show that the vagus carries such secretory fibers to the kidney, while others attribute such to the splanchnics.

The difficulty in reaching a conclusion on the subject has been in the main due to two causes. In the first place, stimulation or section of vagus or splanchnic causes such marked vasomotor changes that any possible secretory effects are easily overshadowed; in the second place, the operation which attempts the division of the plexus of nerves at the renal pedicle has given inconclusive results because of the uncertainty that all the nerves were cut.

To meet this difficulty it was determined to study the function of a kidney which had been removed from the body and subsequently restored to its normal circulation by means of suture of the blood vessels. In this way it is possible to be sure that the kidney is freed from all nerve control, for a time at least.

The results of this portion of the work have been reported in the Journal of Experimental Medicine for April, 1916.

By transverse incision a dog's kidney was made accessible. After being surrounded by silk cloths, impregnated with liquid vaseline, the structures forming the pedicle of the organ were cut, and the kidney removed from the body. A suture of artery, vein and ureter was then made, by which the circulation was restored after an interruption of about one hour, At varying periods after this operation the function of the kidney was studied by comparing it with that of the intact kidney.

The result obtained from this series of observations showed that the function of the reimplanted kidney was greater than normal during the days immediately following operation until about two weeks had passed, when it was found normal.

These observations, together with the fact that it has been repeatedly possible for a single reimplanted kidney to maintain normal existence of the dog after removal of the kidney on the other side, tend strongly to exclude the existence of any specific secretory nerve fibers.

In this present communication the action of diuretics on such a reimplanted kidney has been studied. As examples of the three types of diuretics, sodium chlorid in hypertonic solution, urea, and caffein

or diuretin were used. It is possible that such diuretics as do not cause increase of general blood pressure may act by stimulation of secretory nerves if we suppose such to exist.

In order to register the action of the injected substances the kidney was placed in an oncometer of the type designed by Roy, and by it changes in the volume of the organ were recorded on the kymograph. Observations of flow from the cut ureter of this kidney were also made.

A series of observations was first recorded from normal animals, after which the same method was applied to those bearing a reimplanted kidney.

It was found that the type of reaction shown by the normal and reimplanted kidney is practically identical for the same diuretic.

Here again, then, as in the series of observations already reported, we are unable to find any evidence of abnormality on the part of the reimplanted kidney, its response to diuretics being the same as that of the intact organ. In other words, the reimplanted kidney shows no evidence of loss of any possible secretory nerve influence.

On the movements of the isolated ureter of the dog. GEORGE B. ROTH.

It was found that longitudinal segments and strips, as well as ring preparations of the kidney-, middle- and bladder-third of the isolated ureter of the dog could be made to beat rhythmically for many hours when placed in oxygenated Locke's solution which had a H-ion concentration of 8.3.

Two types of contractions were seen in most experiments, namely, large and small. These types were produced not only by segment preparations but also by the strip preparations in which the circular fibers were cut, so that both types are apparently due to the longitudinal fibers. The large type contractions consume about one and one-half to four seconds in contracting and from seven to seventeen seconds in relaxing. The small type may be more sluggish and sometimes require six to eight seconds for contraction; relaxation requiring twenty to thirty seconds. The small type contractions predominated in the kidney-third; while in the middle- and bladder-third the large type were most frequently found. Relaxation which was independent of contraction was seen in a few experiments in which kidney segments were used.

The rate of the contractions in the various parts of the ureter varies from 4 to 50 in ten minutes. In any segment the contractions are relatively infrequent at first and later become more frequent. The amplitude of the contractions also varied; as a rule segments from the middle- and bladder-third contracted more vigorously than those from the kidney-third.

Epinephrine and barium were shown to stimulate all parts of the ureter. This was shown by an increase in the tonus, rate and amplitude of the beating segments. After apocodeine the epinephrine effect is abolished or lessened, while the response to barium is practically normal, thus indicating that autonomic nerve endings are present throughout the ureter.

Apocodeine first stimulated then depressed ureteral activity. Cocaine and novocaine produced stimulation.

Monobasic acid sodium phosphate caused depression of ureteral movement, which could be counteracted by the addition of small amounts of sodium bicarbonate.

The stimulating action of the bromide-ion on smooth muscle. THEO. K. KRUSE.

Experiments were made on isolated strips of intestines of the cat and dog. Other types of smooth muscle were also studied such as the oesophagus, stomach, intestines of a frog and turtle and of the oviduct of a turtle. The usual suspension technic for smooth muscle was used. The chloride salts of a Ringer's solution were replaced by the corresponding bromide salts. The action of a chloride and a bromide Ringer's solution on smooth muscle was compared.

The results of the investigation may be summarized in the following manner:

1. The action of the bromide-ion is stimulative in nature. This stimulation falls on tone, amplitude and rate. The change in tonicity is the more constant reaction.
2. When two strips are set up at the same time in each solution, the one in the bromide solution usually responds first.
3. With no preëxisting rhythm in a chloride bath, rhythmicity is frequently induced by changing the strip to a bromide bath. If at this time, it should be changed back to the chloride bath, the rhythm may again be abolished.
4. A strip of low or decreased activity responds with greater tone changes than does a rhythmic strip. This seems to be associated with tissue oxidation.
5. Frogs are less responsive to these reactions than are the mammals.
6. Frequently a regularizing influence is observed during the bromide application.
7. An attempt has been made to associate these reactions, in part, to changes in tissue respiration.

The physiology of the chromatophores of fishes. II. Responses to alkaline earths and to certain neutral combinations of electrolytes. R. A. SPAETH.

In dilute sea-water the melanophores of *Fundulus* remain expanded for some hours. The individual salts of sea-water, however, with the single exception of NaCl, cause a contraction of the melanophores. The effects of various combinations of the salts of sea-water and the chlorides of Sr and Ba have been studied.

$CaCl_2$ is a less effective contracting stimulus than is KCl both in pure solutions and combined with NaCl. In comparing the effects of $CaCl_2$ and NaCl upon the KCl contraction it appears that $CaCl_2$, itself a contracting salt, increases the time of the KCl contraction more than does an equivalent amount of NaCl. The addition of a relatively small quantity of $CaCl_2$ to a contracting mixture of NaCl and KCl (Na: K = 5: 1) prevents the normal contraction. Since the normal

contraction is undoubtedly due to KCl, the $CaCl_2$ inhibits the action of the KCl. If the $CaCl_2$ is added after contraction has occurred, the melanophores promptly expand. Thus, in this case, the addition of a contracting salt actually produces an expansion of the melanophores. $CaCl_2$ inhibits the KCl effect only to an optimum concentration. The addition of further $CaCl_2$ produces a more and more rapid contraction. A comparison of the inhibitory action of $CaCl_2$, $MgCl_2$, and $MgSO_4$ shows that they are effective in the order named.

Comparative studies upon the effects of the chlorides of Ba, Sr, Ca and Mg have shown that as regards the speed of the initial contraction, the liminal concentration for the inhibition of the KCl contraction, the time required for complete recovery and the toxicity, the order is

$$Ba > Sr > Ca > Mg$$

The recovery from Ba is peculiar in initiating a pulsation of the melanophores. By means of the methods described in previous publications, all of the above results have been graphically represented and will appear shortly in a detailed publication.

The absorption of fat in partially and in completely depancreatized dogs. CHARLES W. MCCLURE, BETH VINCENT and JOSEPH H. PRATT.

It was found that dogs with a subcutaneous pancreatic transplant secreting and discharging pancreatic juice externally absorbed no more fat than a dog in which the pancreatic remnant was undergoing rapid atrophy and sclerosis. Hence, the condition of the pancreatic tissue remaining in the body does not influence the amount of fat absorbed by the intestine.

The absorption of fat by the intestinal mucous membrane is always markedly disturbed when the pancreatic secretion is excluded from the intestine.

After the complete removal of all pancreatic tissue from an animal, the absorption of considerable amounts of fat can still take place.

Absorption experiments were made on three completely depancreatized dogs. The fat absorption ranged from 17.6 per cent to 45.6 per cent; the amount absorbed from 19.8 grams to 179.4 grams; the amount per kilo of body weight from 1.7 grams to 17.9 grams.

The visible structure of cell protoplasm and death changes. ROBERT CHAMBERS, JR.

1. This paper deals with results obtained on dissecting living cells under high powers of the microscope by means of fine glass needles held in Barber's mechanical pipette holder.

2. Protoplasm is a hydrophyllic colloid which, in cells such as early germ cells, egg cells and protozoa, usually exists in the sol state with a surface layer in the gel state. Adult somatic cells generally are gels in which one can not demonstrate a cell membrane possessing a consistency different from that of the cytoplasm within.

3. The microscopically visible granules in the cytoplasm of the egg of Arbacia may be classified into two types: (a) The microsomes, which are considerably less than 1 micron in diameter and constitute the most resistant parts of the cell maintaining themselves after complete disorganization of the cell, (b) the macrosomes, which range from 1 to 4 micra in diameter and are extremely sensitive to injury.

4. The external surface of the egg cell is a gel which passes gradually into the sol in the interior. The surface gel is very extensile and contractile and is readily regenerated on injury. Tearing of this surface, if unrepaired, results in the pouring out of the internal cytoplasm and dissolution.

5. A remarkable property of protoplasm is its ability to form a protective gel film not only on its external surface but also around an injured area which is in the process of disorganization. The disorganized mass thus insulated is eventually expelled from the cell.

6. A continuous but gradual application of mechanical injury can be sustained by a cell for some time without evidence of harm done. A short but rapid application produces instant local destruction, the spread of which may involve the entire cell.

7. Injury is accompanied by a swelling and an apparent increase in the acid reaction of the part involved.

8. Disorganization of the cytoplasm of the egg cells studied takes place in the following way: first, the megasomes swell and go into solution; and second, the liquid hyaline cytoplasm may flow out and disappear in the surrounding water or it may suddenly set, forming a rigid coagulated mass. The coagulation structure gradually coarsens with the production of a network or granular precipitate.

The effect of α-amino acids and peptones on the growth of cells in vitro. CLARENCE A. NEYMANN and MONTROSE T. BURROWS.

The observations recorded were noted during a series of studies directed towards finding an artificial medium for cultivating cells *in vitro*. In the tissue culture the nutrient materials for the growing cells are derived from the cells disintegrating in the centre of the fragment of tissue. The culture reaction is none other than a transfer of materials from these cells in a less favorable environment to the growing cells on the periphery of the fragment which are in a more favorable environment. Previous evidence had given rise to the belief that the body cells are fluid-like structures. They are unable by themselves to break down complex substances and to build the products thus liberated into their own protoplasm. Growth in these cells is a reaction between themselves and a specifically organized mechanical and chemical environment. It is a differential surface tension reaction. There is a definite period for the growth of cells about a given fragment which has been placed in a medium of blood plasma. Transplanting fragments every three days, we can predict the number of transplants in which growth may be observed. This differs with different tissues.

We tried the effect on this reaction of the addition of carbohydrates,

fats, peptones and *a*-amino acids to the medium. Fragments of heart muscle and skin of chick embryos and foetal chickens were the tissues used. They were planted in plasma, prepared from the blood of adult chicken. The addition of carbohydrates and fats had no effect of present interest. The addition of a 2.5 per cent isotonic solution of peptones prepared from the yolk of eggs had no effect. The addition of an isotonic solution of a mixture of *a*-amino acids, prepared by hydrolizing egg yolk proved toxic. Fearing this toxicity might be due to the method of preparation we tried the addition of isotonic solutions of the individual *a*-amino acids. These have also proved to be toxic when used in certain concentrations. The cells of young embryos are killed after a few days' growth in the first culture. The cells of older embryos and foetal chickens are more resistant but they are killed after a few transplants. Lower dilutions also inhibit the growth and eventually kill. At first, however, they show slight stimulation. This is a functional rather than a growth stimulation. Fragments of heart muscle were used. The stimulation is noted by the active contraction of the fragment.

Muscular Irritability. C. C. GUTHRIE.

The studies were made on skeletal muscle-nerve preparations of frog, and heart tissues of frog and terrapin. Possible technical errors were controlled.

Augmentation. Strong stimulation increases response of skeletal muscle to subsequent single stimuli. In magnitude, the increase may be hundreds of per cent. The period of increased response varies with strength and degree (rate, and strength of stimuli), and with the state of the preparation. The phenomenon is evoked only when certain relationships between state of tissue, and strength and rate of stimulation are realized. The effects are obtained when all stimuli are applied either to nerve or muscle, or alternately to the two, i.e., tetanizing muscle directly and then stimulating nerve with single stimuli, or *vice versa*. Other evidence that nerve terminations may contribute to the effect was presented.

Depression. Stimuli applied to heart during the absolute refractory period may cause an extension of the refractory period. If of suitable strength, and if such stimuli be repeated at short intervals, a further marked extension of the refractory period may be produced. If stimulation be discontinued for a short time and then a single stimulus applied, the muscle contracts. This phenomenon may be observed on isolated ventricular or auricular tissue and succeeds after atropin, so it seems to be independent of vagus action. Studies with natural impulses were included. High frequency current effects on skeletal muscle give evidence of an extension of refractory period. Interpolated single stimuli also depress, but quantitatively the effect is small.

Discussion. Underlying fundamental processes concerned in augmentation are, no doubt, of chemical and physico-chemical character. Such processes may result in increased irritability; increased contractil-

ity; slowing in muscular relaxation; participation of more contractile elements, due (a) to increased irritability, (b) facilitation of conductivity; facilitation of summation; a participation of two or more of the above factors.

A factor probably concerned in the differences observed in augmentation is the greater preponderance of depressing fatigue effects in certain instances. It seems reasonable to infer that augmentation of fundamental processes, although present, may be obscured by such fatigue influence, since our method of judging augmentation is the algebraic sum of two or more effects. Depressing influences more directly associated with stimuli also must be considered. But in general, it seems best to consider that the differences in the factors are more of degree than kind. Factors latent in certain conditions may become manifest under other conditions. Depression is probably best interpreted by giving major consideration to extension of refractory period. A tentative classification of the phenomena was suggested.

Studies in muscular power and fatigue. A. H. RYAN and J. H. AGNEW.

The work reported (on man) consists of (a) a new instrument for analyzing the tension changes occurring when a muscle is contracted either in shortening or isometrically; (b) the application of the results obtained to a critique of previous methods; (c) a new machine for measuring muscular power; (d) results.

(a) The instrument consists of a pair of steel springs arranged on a frame, in such a way that the muscular contraction produces an expansion of the springs which moves a lever over a row of brass contacts set in fiber, closing electric circuits. Each contact is accurately calibrated so that the tension curve can be determined.

(b) Tension multiplied by time has been taken as the index of muscular performance. When the tension-time curve is compared with the work curve in the Mosso method, the former is seen to undergo an irregular increase as the latter decreases. Minute analysis of the individual curves shows why this must necessarily occur as fatigue comes on. Such analysis also casts doubt upon the importance of devices for unloading the muscle during relaxation, also on the interpretation of results obtained with Mosso's ponomètre. The Treves and isometric spring methods were also studied. Mosso's method is considered to have essential faults in conception, while objections are raised to the other methods investigated.

(c) The new instrument is constructed from entirely different conceptions. Tension-time is taken as the index of muscular performance, but the muscular performance has been unified by an arrangement in which the weight decreases as the muscle shortens, in a manner corresponding to the decrease of contractile stress of the muscle (principle of Fick's cosine lever). The end-point is definite, and unpleasant sensations of fatigue are absent. Certain associated unconscious movements were also measured, for the purpose of throwing some light on the course of the voluntary effort under different conditions.

(d) Great normal variation in muscular power was seen. The performance was frequently greater in the evening than in the morning, and frequently very high after a loss of rest. Control subjects were used in studying the effect of barometric pressure, and it was found that their curves followed different courses; and that no relation could be established between barometric level, or direction, and muscular performance. The performance under caffein was much greater than the low or average level, but no greater than the high level of normal muscular performance. The greatest caffein effect was observed four to six hours after administration of the drug. Results obtained in the associated movements will be reported later.

A comparison of the amount of catalase in the muscles of active and inactive animals. W. E. BURGE.

A comparison of the catalase content of the leg muscles and back muscles of active wild rabbits and inactive tame rabbits, of active gophers and inactive rats, of active ground hogs and inactive dogs, was made. A comparison was also made of the catalase content of the leg and breast muscles of active wild pigeons and inactive bantam chickens. After these animals had been washed free of blood by the use of large quantities of 0.9 per cent sodium chloride the muscles of which the catalase content was to be determined were removed and ground up in a hashing machine. One gram of the material was added to 45 cc. of hydrogen peroxide in a bottle, and as the oxygen gas was given off it was conducted through a rubber tube to an inverted burette previously filled with water. After the gas thus collected was reduced to standard atmospheric pressure the resulting volume was taken as a measure of the amount of catalase in the gram of muscle.

The average amount of oxygen liberated by 1 gram of the breast muscle of six pigeons was 91 cc., the average amount of six bantam chickens, 7 cc. By comparing these amounts of oxygen it will be seen that 1 gram of the breast muscle of the pigeon contains about thirteen times as much catalase as that of the breast muscle of the bantam chicken. The pigeons were able to fly practically indefinitely while the bantam chickens could scarcely fly at all. A comparison of the catalase content of the corresponding muscles of the other animals showed that the active muscles were richer in catalase than the inactive muscles, but there was not such a marked difference in any of the muscles compared, as between the breast muscles of the bantam chickens and the pigeons because there was not so much difference in the amount of work done.

A study of the total and preformed creatinine in various muscles of the cat. ERNEST L. SCOTT and ADELAIDE SPOHN.

Cats were used throughout the work. They were killed by decapitation. The muscles were then removed and cleaned of connective tissue and fat either at once or after they had been fatigued. The determinations were made by the methods described by Folin.

1. Do the several muscles contain specific amounts of creatine and creatinine? The muscles studied were the diaphragm, the extensor longus digitorum, the soleus and the bladder. The results obtained from a series of 23 animals indicate that each muscle contains a specific amount of creatine. Judging from the bladder the amount of creatinine in smooth muscle is different from that of striated muscles though these latter do not differ among themselves.

2. Does fatigue bring about a change in the amount of creatine or creatinine in an excised muscle? (a) Ten cats were killed and their extensors were removed. One from each cat was fatigued to exhaustion in a moist chamber by induced electric stimulation while its mate was hung in a moist chamber under similar conditions, but without stimulation. After the stimulated muscle was exhausted the total creatinine was determined in each. Four pairs of solei were treated in a similar manner. The results obtained were alike for the fatigued and resting muscle within the limits of error inherent in the method. Similarly there was no change found in the amount of preformed creatinine after fatigue under the same conditions.

3. Are the effects of fatigue upon the muscles of the intact animal the same as on the excised muscles? Three cats were decapitated after severe fatigue brought about by running in a revolving wheel. The total creatinine was determined as before. The results are shown in the last column of the table and indicate a loss of from 18 per cent to 30 per cent. That this loss extends to the bladder as well as to the muscles which were more obviously working is not contrary to recognized principles of fact and is, we think, capable of explanation.

	CREATINE + CREATININE; GRAMS PER 100 GRAMS OF MOIST TISSUE; RESTING MUSCLE	PREFORMED CREATININE; GRAMS PER 100 GRAMS OF MOIST TISSUE; RESTING MUSCLE	CREATINE + CREATININE; GRAMS PER 100 GRAMS OF MOIST TISSUE; ANIMALS FATIGUED IN WHEEL
Diaphragm	0.368 (23)	0.0044 (20)	0.288 (3)
Extensor	0.427 (23)	0.0048 (10)	0.324 (3)
Soleus	0.278 (23)	0.0042 (10)	0.228 (3)
Bladder	0.097 (10)	0.010 (9)	0.068 (2)

The cardio-skeletal quotient. W. L. MENDENHALL.

By means of faradic quantitative stimulation the relation between strength of stimulus necessary to excite contraction in the frog's ventricle and that necessary to excite contraction in the frog's gastrocnemius was investigated.

The heart was stopped by means of a ligature and the threshold was determined by means of electrodes which were thrust directly into the ventricular wall. The threshold of the gastrocnemius was determined by means of the same electrodes thrust directly into the belly of the muscle, the skin having been previously slit. The gastrocnemius was not curarized.

The threshold value of the gastrocnemius was divided by the thresh-

old value of the ventricle. This ratio was found to be 0.17, and the name cardio-skeletal quotient was suggested for it. According to the evidence obtained the cardio-skeletal quotient is a body constant and indicates that the threshold of the frog's ventricle is approximately six times as great as the threshold of the gastrocnemius.

The use of the cardio-skeletal quotient as an index of the effects of physiological or pharmacological agents was indicated. An investigation using the quotient as indicated was reported. With the gastrocnemius kept in a constant or normal condition, an increase in the cardio-skeletal quotient would indicate an increase in the irritability of the ventricle: conversely a decrease in the cardio-skeletal quotient would indicate a decrease in the irritability of the ventricle. Accordingly it was shown that fatigue causes an increase in the irritability of the heart from 15 to 30 or 40 per cent, varying with the time which elapses after the electrodes are placed in the heart. It is greatest about forty-five minutes or an hour after the fatiguing process has been stopped.

Instances were reported wherein drugs caused decided changes in the irritability of the ventricle as shown by the cardio-skeletal quotient ranging from 0.04 to 0.76.

Hunger and appetite in fever. J. R. RUPP, J. MEYER and A. J. CARLSON.

In experimental fevers induced in healthy persons by the injection of sodium nucleate or excessive doses of typhoid vaccine, the gastric tonus and hunger contractions remain normal unless the body temperature exceeds 104°F., in which case they are depressed. In the fevers ranging from 101° F. to 104°C., the normal gastric hunger contraction may not give rise to the sensation of "hunger" but to an epigastric discomfort tinged with nausea, or "sick stomach." During the chill stage of the fevers, the gastric tonus and hunger contractions are depressed.

In experimental (non-infectious) fevers in dogs, the gastric hunger contractions remain normal, at least until the body temperature exceeds 104° F., when depression usually acts.

Bacterial toxins (exotoxins) or solutions of bacteria (endotoxins) appear to have no depressor action on isolated strips from the stomach wall.

The influence of temperature on the gastric hunger contractions of some of the lower animals. T. L. PATTERSON.

Within certain not very wide ranges of temperature the hunger movements of the frog's stomach fall within the limits of van't Hoff's rule. The hunger contractions of this animal are completely inhibited at 13°C. for low temperature and at 35°C. for high temperature. Raising the first temperature or lowering the second 0.5° or at the most 1° starts the hunger contractions again. In both the frog and the turtle the gastric hunger activity is proportional to the climatic changes in temperature within certain limits as is indicated by van't Hoff's rule.

Further observations on cerebral heat centers. ERNEST SACHS and P. P. GREEN.

Previous experiments stimulating the caudate nucleus faradically showed no temperature changes. Since then 93 experiments on cats and rabbits have been carried out. This paper also includes observations on over 150 craniotomies in human beings. There were 31 electrolytic lesions, 16 injection experiments, 40 irrigation experiments after the method of Barbour. In the electrolytic lesions, temperature rises were noted, but controls showed just as much rise. Injection of emulsion of cortex and caudate into the caudate showed similar temperature changes, but controls showed the same. The results of irrigation by the Barbour method were very variable and a rise of temperature with cold water and a fall with hot was not observed. Irrigation of the liver, using this cannula, gave similar results.

These experiments do not confirm the view that there is a cerebral heat center.

An application of Boyle's law and Avogadro's law to the oscillations of the manometer in clinical measurements of blood pressure. A. M. BLEILE.

Erlanger[1] has put forth an hypothesis bearing on the clinical measurement of blood pressure. This hypothesis practically states that the oscillations of pressure in the arm band, with a given volume pulse change, would be proportional to the pressure of the arm band; e.g., with a given volume pulse change, if the arm band pressure is at 100 mm., the pulse wave shown by the arm band manometer would be *only half as great* as it would with the same volume pulse but with the arm band pressure at 200 mm.

Upon testing this hypothesis by the help of a suitable physical model it is demonstrated that such is not the case. On the contrary, it is demonstrated that oscillations of volume occupied by given mass of gas produce inversely proportional oscillations of absolute pressure.

Or, in other words, the absolute pressure of a given mass of gas is inversely proportional to its volume.

In short, it is another way of stating Boyle's law, that the absolute pressure of a gas is proportional to its concentration.

Therefore, the results of the present work are in harmony with Boyle's law but are contrary to Erlanger's hypothesis.

The influence of an oxidizing substance on the catalase value of the blood. AARON ARKIN.

In previous work the author has demonstrated that certain oxidizing substances (sodium iodoxybenzoate) stimulate the production of antibody in immunized animals, as was shown by Hektoen for dogs immunized with ox corpuscles. The author also found that the above substance inhibits the tuberculin reaction in tuberculous animals, as shown by Amberg for other local allergic reactions, and concluded that the stimulating effect on antibody production was due to a stimulation

[1] Erlanger: This Journal, 1916, xxxix, 401.

of oxidation at the sites of antibody production. In an attempt to analyze this action a study has been made of the effect of oxidizing substances on the catalase value of the blood and tissues. With the assistance of E. B. Fink I have found that sodium iodoxybenzoate does not affect the catalase value of the blood of rabbits. Other oxidizing agents studied by me have no effect. The study of the effect on the tissues of immunized animals is now under way.

The vaporization of water from skin and respiratory passages in health and disease. EUGENE F. DU BOIS and G. FABIAN SODERSTROM.

The water elimination has been determined in 300 experiments made on patients and normal controls in the Sage respiration calorimeter. All observations in which the relative humidity changed more than 10 per cent in three hours have been excluded from the averages since large amounts of water vapor can be absorbed or given off by the bed and clothing with a changing humidity. This leaves 175 experiments made under standard conditions, the air temperature between 22 and 24°C.; relative humidity, 25 to 50 per cent; and the subjects dressed in shirt, pajamas and two pairs of socks.

Thirteen normal men gave off on the average 29 grams of water an hour and 24 per cent of the calories were lost through the vaporization of water, with a range of plus or minus $\frac{1}{10}$. Average results very close to this figure were given by men after large meals of glucose or protein, boys twelve to thirteen years old, very old men, men with legs cut off, dwarfs, patients with pernicious anemia, cardio-nephritic disease and diabetes. The percentage of heat lost through vaporization was increased in typhoid patients with falling temperature and in a few cases of hyperthyroidism.

Some physiological disturbances induced in animals by nitrobenzol fumigation. M. DRESBACH and W. L. CHANDLER.

This paper deals, as a preliminary report, with the effects of nitrobenzol on animals when it is given by inhalation, under conditions in which disturbing factors, such as impurities, CO_2, excessive moisture and temperature variations are reduced to a minimum.

Nitrobenzol acts mainly on the central nervous system and blood. The present study is concerned with the former only. The symptoms vary with different species of animals and with individuals of the same species. In rabbits, cats and rats the dominant symptom is depression and paralysis; in frogs these symptoms alone are seen; in dogs the action is more complex. Rabbits and guinea pigs are very resistant to the poison, but they succumb quickly once the symptoms develop. Dogs may show signs of poisoning within a few hours after breathing the vapor, or not until several (even four) days after the inhalation. The time will depend upon the amount of the benzol taken in. One of the first effects is vomiting. This is followed soon by loss of coordination of muscles. The hind legs are the first, usually, to be affected; then follow the fore leg muscles and those of the neck, jaw and trunk. Ex-

tensor rigidity is the most prominent condition; opisthotonus may be very marked. The attacks may last variable lengths of time, some times for hours. In dogs and guinea pigs typical "swimming" movements are often seen, persisting for shorter or longer periods. In dogs there is practically always nystagmus, and we have seen other evidences of cerebellar stimulation. Likewise, there is dilation or contraction of one or both pupils. Consciousness is present till a late stage. Digestion is interfered with from the start and the animals usually refuse food and drink, though they can often be persuaded to take liquids between attacks of the spasms.

Recovery may take place within a week or ten days following the onset of the intoxication, or the animal (dog) dies, apparently from respiratory failure. We have not seen the circulation fail first.

The experiments so far made indicate that nitrobenzol stimulates and then paralyzes motor centers of skeletal muscles. The circulation and respiration in dogs are but little affected, unless violent convulsions ensue. The effects upon the blood have not been sufficiently studied, but it is known that profound changes in the oxygen carrying capacity and in the morphology and number of the different corpuscles may occur.

Physiological effects of ethyl alcohol when injected into the rectum with special reference to the gaseous exchange. T. M. CARPENTER.

The respiratory exchange of four healthy medical students before and after injection of alcohol into the rectum was studied by means of a face mask and the Tissot respiration apparatus. For control studies 0.6 per cent sodium chloride solution was used. The composition of the expired air was determined by the portable Haldane gas analysis apparatus. Simultaneous graphic records were secured of the pulse and respiration rates as well as of the extraneous muscular activity. The concentrations of alcohol injected were 5.0, 7.5 and 10 per cent. The amounts given were from 200 to 750 cc. Usually the absolute weight of alcohol was 25 grams.

When the respiratory quotient was about 0.85 before 25 grams of alcohol was given, it fell to 0.80 two to three hours after the injection; when the respiratory quotient was 0.80, it fell to 0.77 in about the same length of time. This depression of the respiratory quotient did not occur in all cases. In a few experiments the oxygen consumption was raised about 10 per cent within three hours after the alcohol. In several instances the pulse rate increased from three to five beats per minute over that of the period before alcohol was given.

Reflex control of gastric vagus-tonus. F. T. ROGERS.

Dogs, in which the splanchnic nerves have been sectioned and with gastric fistulae, may show hunger contractions after decerebration (Sherrington's method) following a fast of two or three days. Transection of the brain stem must be done under *deep* anesthesia. Unless the dog again breathes normally and unless the pulse rate and strength

are nearly normal, the hunger contractions will not appear. Respiratory or cardiac disturbances indicate a quiescent stomach. Pressure effects on the medulla do not seem to stimulate the gastric centers.

Carlson has called attention to the fact that the hunger contractions are very easily inhibited by various reflexes but in no case is there a reflex augmentation of these contractions or of gastric tone.

In a dog, prepared as indicated above, stimulation of the central end of one vagus nerve with the other nerve intact, is followed by reflex spasmodic contractions of the entire stomach. This effect may be obtained by stimulating the nerve either in the neck or below the diaphragm. In the absence of the splanchnic nerves reflex augmentation of gastric tone and contractions is possible. The vagus contains both the sensory and motor paths of this reflex. This suggests that vagus tonus, with reference to the stomach, may be a reflex effect similar to that of muscles.

An instance of apparent anesthesia of a solution. E. NEWTON HARVEY.

Luminous bacteria will cease to light in definite concentrations of an homologous series of alcohols, and if the alcohol be removed or diluted light again appears. The concentrations which narcotize in one hour are: methyl alcohol, 2 m.; ethyl, 1.5 m.; propyl, 0.25.; butyl, 0.08 m.; amyl, 0.03 m.; capryl, 0.002 m. The series is similar to that found in narcosis of other cells and brings the anesthesia of light production in line with that of muscle contraction and other physiological processes. The preparation of luminous extracts of luminous bacteria is practically impossible. Many luminous animals, however, secrete luminous substances; the small ostracod crustacean, *Cypridina*, forms a brilliant clear luminous secretion whose light is perfectly homogeneous. The secretion contains both photogenin and photophelein, the substances necessary for the production of light, and it may be filtered through a porcelain or siliceous filter candle and is then still luminous. The light producing substances are therefore in true or colloidal solution. If ethyl alcohol is added to the porcelain filtrate, the light disappears when about 20 per cent alcohol is reached and on diluting, the light returns. With butyl alcohol, less than 8 per cent is needed. As no precipitate forms, we are not dealing with a precipitation phenomenon, but with a reversible inhibition of light production in a solution akin to anesthesia.

Minimal variations in spinal reflex thresholds. EUGENE L. PORTER.

The object of this investigation was the study of and control over some of the variables encountered in measuring reflex thresholds in the spinal cat, and in particular the variations in threshold of the flexion reflex were to be compared with those of the nerve muscle preparation. Spinal cats had tibialis anticus muscle arranged for recording myographically, when reflex contraction was elicited by stimulation of the posterior tibial nerve; subsequent stimulation of the distal end of the

cut peroneus nerve gave direct nerve-muscle records from the same muscle. A modification of Lucas' liquid electrode was used in place of the platinum wire type, to avoid local impairment of the nerve from compression, and to prevent alterations in resistance at the point of stimulation due to the presence or removal of liquid. This electrode consists of a short glass tube, constricted at one end, into which the ligated nerve is drawn; over the other end is pushed a rubber tube, whose lumen 1 cm. from the glass tube is closed by a glass ball through which passes a platinum wire. Within the glass and rubber tubes the nerve is surrounded by a thin paste of kaolin in Ringer's solution. Induction shocks sent into the platinum wire spread through the kaolin-Ringer paste, stimulate the nerve at the constricted neck where current density is greatest, and leave the animal at an indifferent electrode on the fore-limb. Determinations of threshold were made approximately once a minute for periods an hour long, using a coil calibrated by Martin's method. During an hour both the reflex and nerve-muscle preparations were found to vary as much as 2 Z-units but at the end the threshold might be either lower or higher than at the beginning. Alterations of threshold between successive readings were from two to three times as great for reflex as for nerve-muscle, and the direction of change toward a higher or lower threshold was reversed more often for reflex than for nerve-muscle. Comparisons of flexion with crossed-extension showed that flexion thresholds varied less than those for crossed-extension.

Possible periodic variations in the extent of the knee-jerk in women. JESSIE L. KING.

Repeated attempts have been made to demonstrate by physiological and psychological tests that the life-processes of women have a rhythmical movement which centers about the menstrual period. Except as concerns the slight constant variations in pulse and temperature, these attempts have failed. Possibly a variation in the tone of the nerve-centers, as well as the rise in temperature may account for the feeling of discomfort frequently experienced by normal women immediately preceding and at the onset of the menstrual period.

Using the knee-jerk as an index of such tone, a series of daily observations was made on a group of eleven normal young women, over periods varying from one to five menstrual cycles. The observations covered twenty-nine periods. A chart showing the daily averages for one subject was submitted.

The evidence pointed in the direction of the following conclusions—that a period of hyperexcitability immediately precedes or accompanies the onset of the menstrual period; that this is followed by a decline in excitability which continues for a few days after the menses has ceased; and that there is then a tendency for it to rise to a slightly higher level than the preceding during the intermenstrual interval.

The combination of thrombin by the antithrombin of the blood serum. H. S. GASSER.

If very clear oxalated plasma is heated to 55° to remove its fibrinogen, the coagulation process may be initiated by optimum calcification and the stages in the conversion of prothrombin be studied quantitatively from the start. If the amount of tissue extract present is so small that all the prothrombin is not converted to thrombin then the amount of thrombin that can be produced is formed rapidly and disappears rapidly, going into combination with antithrombin. Quantitative determinations show that the curve of free thrombin is practically the inverse of the combined-thrombin curve. The system soon comes to equilibrium, no more prothrombin is converted to thrombin, therefore there is no evidence that the action of tissue extract is catalytic. This equilibrium is very readily upset, however, by addition of tissue extract, or by simple dilution. The thrombin in combination with antithrombin is in an irreversible combination; processes liberating the thrombin also destroy the antithrombin. The rapidity with which thrombin is combined by antithrombin explains satisfactorily why thrombin does not cause thrombosis when injected intravenously and is to be regarded as an important agency in maintaining the fluidity of the blood.

The inhibitory effect of adrenalin upon the sphincter muscle of the iris. DON R. JOSEPH.

Adrenalin solutions of various concentrations when applied to the sphincter pupillae after either partial or complete excision of the muscle cause a prompt and unmistakable relaxation. This relaxation is maximal following the use of stronger adrenalin solutions (1: 1000 to 1: 100,000). Very dilute solutions (1:10,000,000) on the other hand, produce only a partial relaxation. The duration of the effect varies directly with the concentration of the solution used; after 1: 20,000,000 recovery may be complete within two to five minutes, whereas after 1: 1000 two hours or more may be necessary for recovery. The sphincter muscle of the following irises was tested: bovine, swine, sheep, goat and human. The changes in sphincter-length were recorded graphically.

These experiments demonstrate that the sphincter muscle of the iris is capable of responding to a suitable stimulus in a sense that is exactly the opposite of shortening (contraction), namely by lengthening (relaxation). In its response to adrenalin the sphincter of the iris resembles the intestinal strip.

The transformation of the plasma clot. GEORGE A. BAITSELL.

It has previously been shown by the author[1] that in tissue cultures and in wound healing in the skin of frogs a fibrous tissue which gives evidence of being identical with normal connective tissues may be

[1] (a) Journ. Exper. Med., 1915, xxi, 455–479; (b) Journ. Exper. Med., 1916, xxiii, 439–456.

formed by a direct transformation of a plasma clot. In an endeavor to analyze this reaction more fully, plasma clots made from centrifuged blood plasma of the frog have been subjected to various conditions of tension and pressure. The results obtained show that with the aid of these mechanical factors it is possible to directly transform a typical fibrin net into a fibrous tissue. By varying the conditions it is possible to obtain preparations which will show various stages in the transformation ranging from an almost typical fibrin net to a fibrous tissue composed of bundles of wavy fibers such as are characteristic of various types of normal connective tissues. This newly formed fibrous tissue when stained with Mallory's connective tissue stain (modified according to Mall) takes the typical blue color the same as do regular connective tissues.

Changes in the percentage composition of muscle protoplasm during prolonged fasting with work. C. W. GREENE.

The Pacific salmon, Oncorhynchus tschawytscha, was used for the determinations presented in this series of experiments. This species of salmon, as is well-known, feeds in the ocean until maturity and then makes the journey to the spawning grounds in cold fresh water streams wholly without eating, a natural fasting period. The journey takes three to four months, and there is expenditure of a great amount of muscular energy during the migration. The source of this energy has been traced by the method of quantitative chemical analyses of the great classes of substances constituting the muscle tissue.

The salmon stores its fat among its muscle fibers and within certain classes of fibers. This storage fat amounts to from 15 per cent to 20 per cent of the wet weight of the muscle, in certain particular muscles as much as 30 per cent. At the end of the migration the excess of fat is reduced to an average of 2 per cent to 2.24 per cent. It has been shown that a large proportion of the gross mass of muscle disappears during the migration fast. The stored fat has been considered as a superload in the determination of the protoplasmic changes. When figured on the assumption that the tissue exclusive of fat may be legitimately considered as muscle protoplasm rather striking compositional changes appear.

Comparing the three collecting regions, namely, the mouth of the Columbia River, an intermediate point at 700 miles up stream, and at the spawning grounds, the following changes are revealed: The water content of the fat-free tissue increases—74.7 per cent, 77.0 per cent and 81.5 per cent, an increase in total water content of about 9 per cent during the journey. The proteins decrease—20.2 per cent, 18.2 per cent and 14.0 per cent, a relative decrease of 30 per cent. The organic extractives increase—2.9 per cent, 3.3 per cent and 3.1 per cent. Migrating fish are most active at the 700 mile station, which doubtless accounts for the higher percentage of organic extractives obtained at this point. The ash remains practically constant throughout the entire cycle of the migration fast.

Further studies on the effect of adrenalin upon muscular fatigue and limb circulation. CHAS. M. GRUBER.

The experiments were performed exclusively upon cats, either under urethane or ether anesthesia. The drop method was used in recording the circulation. The limb was either under normal conditions, except that the nerve was cut and a cannula placed in the blood vessel leading from the muscle under observation or perfused with warm Ringer's solution through the femoral artery.

In all experiments when the nerve was cut, adrenalin slowly injected in small doses 0.5 cc. to 2 cc. of a 1: 100,000 solution produced an increase in the height of muscular contraction, with a concomitant decrease in the rate of flow of fluid through the vessels. This decrease in the intact animal was passive in action, due to the fall in blood pressure. But in the perfused limb small doses 1: 100,000,000 produced active vasoconstriction.

Under experimental conditions in which there is paralysis of the nervous system to the muscular vessels adrenalin does not better fatigue by bettering the circulation, but it betters the height of muscular contraction by its action upon some substances produced during fatigue or upon some substance utilized by the muscle during its contraction.

In a fatigued muscle in which the nerve is intact adrenalin has a twofold effect: that of bettering the circulation, and that, possibly, of direct chemical action similar to that found in a muscle in which the nerve is cut.

During the development of rigor mortis changes take place in the muscle similar to those that take place in fatigue. Adrenalin was found to have a potent action upon muscles undergoing rigor mortis from one to four hours as tested by muscular contraction.

Anti-neuritic substances from egg yolk. H. STEENBOCK.

By means of neutral solvents there was prepared a water-acetone soluble fraction from egg yolk which in small doses by intraperitoneal injections was able to cure a pigeon suffering from polyneuritis. The anti-neuritic principle was found stable to concentrated hydrochloric acid at 98° and to concentrated alkalies at room temperature, but readily destroyed by dilute alkalies at the boiling temperature. Phosphotungstic acid precipitated it very incompletely. It was not adenine.

On the mechanism of blood coagulation. G. H. A. CLOWES.

A suitable citrated plasma filtered through a bougie does not clot on the addition of $CaCl_2$ alone, but if one in a hundred thousand of boiled brain lipoid is added, the filtrate clots with $CaCl_2$ almost as readily as does the original plasma. The filtrate without addition of lipoid clots far more readily with thrombin than does the original plasma, the speed of coagulation being if anything retarded by the addition of lipoid. If brain lipoid is added in excess to such a filtrate without addition of $CaCl_2$ and the mixture filtered a second time, the filtrate obtained does not clot with $CaCl_2$ alone, clots less readily than the first

filtrate with $CaCl_2$ and lipoid and more readily with thrombin. Mixtures of $CaCl_2$ and lipoid or $CaCl_2$ and prothrombin which are toxic for mice on intravenous injection retained their toxicity indefinitely, but a mixture of $CaCl_2$, lipoid and prothrombin in the proportions necessary to cause coagulation exhibits a gradually diminishing toxicity.

The action of brain lipoid is destroyed by the addition of sufficient iodine. Lecithin exerts no effect. Unsaturated lipoids appear to play a positive rather than a negative rôle in blood coagulation.

NaCl at a concentration of 0.35 to 0.4 M destroys living protoplasm in marine organisms or mice, exerts an inhibiting effect upon blood coagulation, breaks down emulsions of oil dispersed in water by Na soap, precipitates soap jellies and soap and lipoid suspensions, and the ratios in which the effect exerted by NaCl and other salts may be counteracted by $CaCl_2$ at varying concentrations appear to be approximately the same in blood coagulation and many of the above systems and to correspond with Loeb's data on antagonistic effects exerted by electrolytes on protoplasm and the writer's observations on interfacial soap films determined by the drop method.[1] The relation between prothrombin and thrombin presents a striking analogy to that between soaps like sodium oleate, which being soluble in water but not in oil promote the formation of emulsions of oil in water, and soaps like calcium oleate, which being soluble in oil but not in water promote the formation of emulsions of water in oil. The consideration of coagulation as a possible reversal of phase relations in plasma under the influence of Ca, analogous to that in emulsions, may offer an explanation for such phenomena as the durability of prothrombin in citrated plasma, its inability to function without calcium and lipoid, the high coagulating effect of the nascent thrombin formed from calcium, lipoid and prothrombin in the presence of fibrinogen, the extremely rapid deterioration of thrombin when present in the water phase, presumably as a result of surface adsorption, its stability so long as it remains a portion of the non-aqueous phase and its ability to function although somewhat slowly in the presence of an excess of citrate.

Recovery experiments following removal of the Sino auricular node in the dog's heart. J. A. E. Eyster and W. J. Meek.

The sino-auricular node was sewed off in seven dogs, in three additional animals it was cut away. The operations were performed aseptically and the animals allowed to recover. Before, and at varying periods of time after the operation, electrocardiograms (lead II) were taken to determine the type of cardiac rhythm. In four experiments, the reaction to muscular exercise and to atropine sulphate before and after the operation was also determined. The experiments varied in duration from 48 hours to 64 days following the operation. In most cases, at the termination of the experiment, the chest was opened and the seat of impulse formation determined by initial negativity with

[1] Journ. Phys. Chem., 1916, xx, 407.

the string galvanometer. Eight of the animals showed periods of auriculo-ventricular rhythm following the operation. In all, however, there subsequently developed a rhythm in which there was a definite, though usually somewhat shortened, auriculo-ventricular interval. In one experiment the seat of the permanent rhythm was definitely within the coronary sinus portion of the auriculo-ventricular node. Others showed the resumption of an apparently normal sino-auricular rhythm, preceded in two by evidences of partial sino-auricular heart block. Histological examinations will have to be made before definite statements can be given as to the relation of the seats of permanent impulse formation to possible remnants of the sino-auricular node.

The perfusion of the mammalian medulla: the effect of carbon dioxide and other substances on the respiratory and cardiovascular centers. D. R. HOOKER and D. W. WILSON.

To determine the effect of various acids and alkalies on the mammalian medulla, perfusion experiments were carried out on preparations similar to those described by one of us in which an isolated circulation is maintained artificially through the head and neck of the animal. Various materials were added to portions of the perfusing blood and the effect on the respiratory and cardiovascular centers noted by recording the movements of the epigastrium and the pressure changes in the femoral artery. In preliminary experiments sodium hydroxide added to blood caused complete inhibition of respiratory movements and hydrochloric acid caused marked stimulation. Sodium bicarbonate caused an increase in amplitude and decrease in rate of respiratory movements. Further experiments were more carefully controlled by determinations of the hydrogen-ion concentrations of the specimens used.

Portions of blood in which the P_H was decreased from 7.4 to 7.2–7.3 by introducing hydrochloric and lactic acids, showed no apparent differences in stimulating action on the respiratory center of the same animal.

Portions of blood through which 5 per cent CO_2 had been passed were compared with blood to which HCl had been added and the free CO_2 shaken out, the resulting P_H being the same in both cases. Alternate perfusions of these solutions through the same preparation resulted in a much greater stimulating action of the blood containing the excess of CO_2. These experiments would indicate a specific action on the part of carbonic acid on the respiratory center.

Observations on immunity of monkeys to experimental poliomyelitis. EDWARD C. ROSENOW and GEORGE W. WHEELER.

Apparent immunity was conferred upon three monkeys by inoculations, which were not followed by obvious evidence of infection. One of the immune animals had previously received an intracerebral injection of plemorphic streptococcus, the second had received emulsions of the central nervous system of rabbits paralyzed by cultures of this

streptococcus, and the third had received virus in the usual way. One monkey injected with green-producing streptococci from sources other than poliomyelitis was not immune, and one monkey injected with virus from monkey which proved not to have poliomyelitis was not immune. Three normal controls developed poliomyelitis in the usual time.

Pulse velocity and blood pressure. An attempt to determine the variations of elasticity in the walls of living arteries. PERCY M. DAWSON.

Many physiologists have come to regard the pulse pressure as an index of the systolic output of the heart (see Dawson and Gorham, 1908). It is however obvious that a pulse pressure of say 50 mm. when the mean pressure is 100 has not the same significance as the same pulse pressure when the mean pressure is 130, unless the coefficient of elasticity of the blood vessels remains constant, which is probably not the case.

Variations of the coefficient of elasticity of the arteries outside the body have been carefully studied (see MacWilliam, 1904, whose observations have been confirmed in great part by Higgins, 1908). But we have no certainty as to the variations which this coefficient undergoes in the living animal. As an index of the variations of arterial elasticity in the living animal one may take the variations in the rate of transmission of the pulse wave. Then if the vessels behave in the body as they do when outside it, one might expect that at the very least a rise in mean pressure would be accompanied by an increase in the velocity of transmission of the pulse wave.

In 1908 the writer, assisted by Lemuel W. Gorham, determined the rate of transmission of the pulse wave in dogs while the blood pressure was being raised and lowered by stimulation of the vagus and of various sensory nerves (central end of vagus and of saphenous). This study showed that the correspondence between the blood pressure and the rate of transmission was by no means close. For example the curve of elasticity would, after rising steadily for a while, in correspondence with the rise in pressure, suddenly fall or begin to oscillate up and down. Consequently if one should determine the rate of transmission for a pressure of say 60 mm., he might find ten minutes later that this value corresponded to an entirely different pressure. These experiments were repeated and confirmed in 1916 by Edwin G. Bloor and Fred J. Hodges, working under the direction of the writer.

The results of those experiments were not however accepted by us because of our lack of confidence in the method of recording. This was by means of two Hurthle manometers which gave curves upon which it was sometimes impossible to identify the beginning of the up-stroke of the pulse wave. It was not until the previous results were again confirmed by experiments in which the segment capsules were employed that publication seemed justified. Such results were obtained by the writer assisted by Hartwik M. Stang and Fred J. Hodges.

We conclude that in so far as the velocity of the pulse rate may be regarded as an index of arterial elasticity, it indicates that in the living

animal the coefficient of elasticity is irregularly variable and it is furthermore suggested that this variability may be due to changes of tonus of the vascular walls.

Does the myelin sheath function as an electric insulator broken only at the nodes of Ranvier? J. F. McClendon.

Many hypotheses have been advanced concerning the function of the myelin sheath. The supposition that it is an electric insulator is not at all new, but may seem out of harmony with the fact that the nerve may be stimulated electrically throughout its length. Through repeated confirmations of the All-or-none Law it has been shown that not all of the fibers in a nerve are stimulated by a minimal stimulus, which suggests the possibility that the current enters and leaves the nerve fiber at a node of Ranvier and that those fibers are stimulated by a minimal stimulus whose nodes are favorably placed. It is often asserted by teachers of physiology that a nerve can not be stimulated by means of a current passing at right angles to the course of the fibers (unless spreading alters the course of the current), which also suggests that the current enters and leaves by the nodes, since more nodes are affected by a current passing lengthwise than by one passing crosswise. In attempting to collect further data on this question the nonmedullated nerves of Limulus were stimulated by minimal currents, and it was found that electrodes the same distance apart, equal stimulation was the result of the current passing crosswise as of the current passing lengthwise, which is in harmony with the hypothesis discussed.

The oxygen pressure necessary for cellular activity. Montrose T. Burrows.

In an early study the author had noted that the oxygen necessary for cellular activity in a tissue culture comes from the air chamber of the hollow ground slide in which the culture is suspended. To reach the cells it must diffuse from this space into the culture medium which bathes them. The cultures were made by placing a small fragment of tissue, 1 mm. in diameter, in a layer of plasma on the surface of a cover glass. The cover glass is inverted over a hollow slide so that the medium and tissue hang in the air chamber. The cover is sealed to the slide with vaseline and paraffine to prevent drying.

In the present study a special type of culture chamber has been constructed. It is so arranged to allow one to pass into it any gas or combination of gases. The air chamber of this culture is large, 40 to 60 times that of the ordinary culture in which growth may continue actively for several days. This culture chamber is made from one piece of glass and it can be sealed by fusing. Tests to determine the effect of pure oxygen and partial pressures of oxygen on the growth of the cells have been made. The partial pressures of oxygen were obtained by diluting pure oxygen with the nitrogen gas. The tissues used have been fragments of heart muscle and skin of chick embryos and foetal chickens. The medium is blood plasma, prepared from the blood of adult chickens.

There is a small error in the measurements of the partial pressure of oxygen.

Including this error the results have been considered interesting. The cells grow actively in pure oxygen. The growth is not greater in an atmosphere of pure oxygen than in an atmosphere containing 9 or 10 per cent of oxygen. A small growth is seen when the partial pressure of oxygen is as low as 6.6 per cent, 45.6 mm. of mercury.

The cause of cataract. W. E. BURGE.

In the production of cataract there are at least two factors, one, the short wave lengths of the spectrum, the other, inorganic salts such as those of calcium, silicon, etc. which are found to be greatly increased over the normal in cataractous lenses. By increasing either of these factors separately far beyond what the eye would ever be subjected to an opacity of the lens or cataract can be produced, e.g., cortical cataract can be produced by immersing the lens in a 5 per cent solution of calcium chloride, and nuclear cataract by immersing the lens in a 15 per cent solution of potassium chloride. By increasing these two factors simultaneously an opacity of the lens or cataract can be produced under conditions that might easily prevail in the body, e.g., if the lens s exposed to the short wave lengths of the spectrum it causes a molecular rearrangement in the protein so that a very weak solution of calcium salts will precipitate it and hence produce an opacity or cataract. In this respect the inorganic salts seem to act in a manner comparable to that of a developer in photography.

Others presented papers, as follows:

The fate of intravenously injected dextrose. J. J. R. MACLEOD.

Primary scrotal syphilis of the rabbit and its utilization in chemotherapeutic experiments (with lantern demonstration). W. H. BROWN and L. PEARCE.

The action of some optic isomers on the ureter. D. I. MACHT.

The time relations of the fundamental heart sounds. CARL J. WIGGERS and A. DEAN, JR.

A comparison of the effects of food and of caffeine on work in the athlete and an untrained subject. C. B. ROOT and H. CURL.

On the toxemia of intestinal obstruction. L. R. DRAGSTEDT, W. BURBY and A. J. CARLSON.

The mechanism of the regulation of the intra-abdominal pressure. HELEN C. COOMBS.

Some considerations of the isolated bone marrow. CECIL K. DRINKER, HENRY A. KREUTZMAN and JOHN R. PAUL.

Labyrinthine reactions in kittens (with demonstration). A. L. PRINCE.

Some observations on the conditions of activity in the adrenal glands. W. B. CANNON and H. F. PIERCE.

Endermic reactions. J. D. PILCHER and T. SOLLMAN.

The active constituent of the thyroid; its isolation, chemical properties and physiological activity. E. C. KENDALL.

The effects of temperature change on rhythm in the human electromyogram. ALEXANDER FORBES and W. C. RAPLEYE.

The so-called experimental streptococcic poliomyelitis (with lantern demonstration). C. G. BULL (by invitation).

The attenuation of the toxic action of KSCN *by elevated temperature.* R. A. SPAETH.

Bacteriologic observations in experimental poliomyelitis of monkeys. EDWARD C. ROSENOW and EDWARD B. TOWNE.

Papers read by title, as follows:

The inorganic composition of thoracic duct lymph. A. B. MACALLUM.

Preliminary report of cardiogram and blood pressure records, showing the effect of music. IDA HENRIETTA HYDE.

The hydrolytic products of chitin. S. MORGULIS.

Demonstrations as follows:

A signal magnet which writes either upwards or downwards. WORTH HALE.

In this magnet, which is useful in recording time intervals, etc., use is made of a double magnet and double armatures. One of these armatures is extended as a writing point, the other serves as an arrest for the recoil produced by the spring when the current is not acting. The armature which thus serves as an arrest is held stationary by its spring against a fixed block. When the electrical current passes to one magnet the writing armature moves in one direction, and when to the other magnet it moves in the opposite direction.

An improved lever for frog's heart and muscle strips. A. H. RYAN.

The essentially new feature in this lever is the use of needle points as bearings. By this method greater sensitivity is obtained and at the same time lost motion is eliminated.

A saddle is made which carries a straw. On one side of the under surface of the saddle is a conical depression, and on the other side is a wedge shaped slit running at right angles to the axis of the straw. The conical depression and slit each rests upon a needle. These needles are mounted in a support which has attachments for two adjustments by which the lever may be made to write parallel to the recording surface; and by which the lever may be made to write with more or less pressure upon the recording surface. The lever may be used for

frog's heart tracings or isolated strips. The detachable arm which supports the strips is hollow to permit of passing gases or liquids into the medium surrounding the strip.

A crude form which could quickly be carved out of cork was also demonstrated.

The inhibitory effect of stimulation of the central end of the vagus nerve upon the contractions of an active respiratory muscle in the chicken. A. L. MEYER.

The innermost abdominal muscle in the fowl when properly exposed is observed to contract during each expiration. It undergoes a sudden relaxation just prior to the onset of inspiration.

The central end of the vagus was stimulated with an induced current. The muscle remained quiescent in a state of relaxation, while the thorax assumed an inspiratory position. This result was obtained many times and without fail during the demonstration. The phenomenon here presented is another instance of "contrary innervation" (Meltzer) or "reciprocal innervation" (Sherrington). This method, it seems, may be useful in demonstrating the subject of contrary innervation to students.

A gas analysis apparatus. YANDELL HENDERSON.

A simple, easily operated and comparatively cheap analyzer was shown, and the methods of determining the composition of the alveolar air, the respiratory metabolism and the blood gases CO_2, oxygen and "oxygen deficit" were demonstrated. While the apparatus is designed primarily for students in a laboratory course it is found to be sufficiently accurate for most purposes of research.

The motion picture as an aid in teaching physiology. J. A. E. EYSTER and WALTER J. MEEK.

Motion picture films were exhibited showing certain typical physiological experiments. It is believed that these are of value as an aid to physiological teaching in the following instances. (1) As a supplement to certain demonstrations. There are certain important aspects of many demonstrations which are difficult to exhibit to a group or class by the ordinary methods. (2) To serve for demonstration of certain experiments which (a) are too long to be carried out in the classroom during the regular class period, (b) in which the typical results are obtained only at certain times under certain conditions, or (c) which are too time consuming or too expensive to involve repetition before each class. (3) To assist in the analysis of certain rapid movements. One of the films shown was that of the exposed beating mammalian heart, taken at a rate of exposure of 80 per second and projected at the normal rate of 16 per second. (4) For demonstrations before elementary classes where the more complex demonstrations involve too much time or expense.

It is not believed that this method will do more than supplement already existing methods of teaching physiological science.

Motor phenomena of the stomach, pylorus and cap observed Roentgenographically. LEWIS GREGORY COLE.

Each portion of the gastro-intestinal tract has a characteristic Roentgenological appearance: The ileum by its coils, the jejunum by its flocculent appearance, the duodenum by its circular rings or its shape, the stomach by its characteristic shape.

That portion of the tract just beyond the pyloric sphincter, previously known as the first or ascending duodenum, has the characteristics of the stomach rather than the duodenum. Embryologically, together with the stomach, it is derived from the foregut. Histologically it contains the glands of Brunner. Ferments from the glands of Brunner are active only in an acid medium; therefore this region is rationally acid. Surgically 95 per cent of ulcers occur in this region. Roentgenologically its contour corresponds with the pyloric end of the stomach, and the manner in which it surmounts the pyloric end of the stomach resembles a cap; therefore the term "cap of the stomach" or "pilleus ventriculi" has been applied to this segment of the gut.

The cap is separated from the pyloric end of the stomach by a clear cut space of about $\frac{3}{16}$ of an inch; this indicates the pyloric valve, and the size of the lumen of the valve can be determined.

A meal composed of mixed foods and fluids begins to be evacuated immediately; certainly before ingestion of a full meal is complete.

The gastric motor phenomenon is complex rather than simple and is composed of the progression of the peristaltic waves pyloruswards and a systole and diastole, and it may be considered in the form of a cycle similar to the cardiac cycle. The stomach assumes a series of shapes and eventually assumes the same shape that it was approximately fifteen or twenty seconds earlier. This is referred to as a gastric cycle. If a peristaltic sulcus travels from its origin to the pylorus during one cycle it is a one cycle type of gastric peristalsis. If it requires two, three or four cycles for a sulcus to travel from its origin to the pylorus this is respectively referred to as two, three or four cycle type of peristalsis. During approximately seven-tenths of each gastric cycle all of the peristaltic sulci synchronously increase in depth; this is gastric systole. During approximately three-tenths of each cycle these sulci synchronously relax; this is gastric diastole. During the systole of each gastric cycle, when the pressure against the proximal surface of the pyloric valve is greatest, the pyloric sphincter is partly opened, either actively or passively, and the chyme is propelled into the reservoir cap; and during the stage of diastole when the gastric pressure is diminished the sphincter may be closed to prevent the chyme from falling back into the stomach.

There is no Roentgenological evidence in man of a periodical opening and closing of the pyloric valve independently of each gastric cycle, as described by Cannon in the American Journal of Physiology, Volume XX, no. 2. If such a phenomenon does not exist, how can it be acid controlled?

The duodenum is replenished from the reservoir cap where the finish-

ing touch of proteid digestion is applied to a small amount of chyme. The cap is evacuated by a broad peristaltic wave, independent of the gastric peristalsis, which forces the chyme through the duodenum in "finger-like masses." The frequency of this wave may be controlled by an alternating acid and alkalin reaction in the descending duodenum.

Demonstration of Nissl preparations indicating an absence of chromatolytic changes in the central nervous system of the woodchuck (Marmota monax) during hibernation. A. T. RASMUSSEN.

The whole problem of the correlation of structural changes in nerve cells with degrees of activity, having been re-opened by the recently reported findings of R. A. Kocher, it is interesting to note the apparent absence of demonstrable changes in the tigroid bodies of nerve cells of animals which have been profoundly torpid almost continuously for months, during which time no food or water was available. Similar results have been reported in other animals, though contrary conditions are also on record.

Killing, fixing and embedding were carried out as uniformly as practicable. Homologous sections from an entire series of animals were placed together on the same slide for staining or an entire series of slides was carried through the reagents in a basket which would contain the entire lot. Two series of animals, involving a total of 21 animals, 9 of which were sacrificed during hibernation, were investigated.

Glycogen in the blood vessels of the liver. G. CARL HUBER and J. J. R. MACLEOD.

Several microscopic slides were shown of sections of liver, removed immediately after death by stunning, from rabbits previously fed with carrots. The pieces of liver after removal from the animal were immediately fixed in absolute alcohol and later embedded in paraffin, cut in sections of 5 mm., and stained by a somewhat modified Best's carmine method. By this means the glycogen is stained a deep red and the nuclei of the cells can easily be counterstained.

Certain of the preparations were from animals that were in a perfectly normal condition up to the moment of stunning. Others were from rabbits in which, three or four hours previous to the stunning, the medulla had been punctured with a needle and the animal thus rendered diabetic. By comparing slides from the two groups of animals it was shown that in normal rabbits the glycogen is present only in the liver cells and never in the sinusoids or sublobular veins, whereas in the livers of diabetic rabbits red-staining glycogen masses are often present in the latter vessels. It is not of course claimed that, because this material in the blood vessels stains exactly the same as the glycogen of the cells, it is necessarily of the same chemical nature.

It was also pointed out by the authors that a very large number of preparations have been examined in order to exclude the possibility that the appearance of the red-staining material in the blood vessels could be due to any displacement of glycogen, caused by the knife

during the making of the sections. Before affirming finally that the migration of glycogen into the capillaries takes place only in cases where hyperglycogenolysis is occurring, as for instance in piqûre, it will be necessary to examine, by the above methods, a greater number of normal rabbits.

Some new apparatus. D. E. JACKSON.

Pathéscope films used to illustrate physiological demonstrations to students. ALEXANDER FORBES.

Photographs representing the growth of chickens fed with definite mixture of food-stuffs under laboratory conditions which have heretofore not led to success. THOMAS B. OSBORNE and LAFAYETTE B. MENDEL.

A convenient form of non-polarizable electrode for class use. THEODORE HOUGH.

A method of recording fundamental heart sounds directly from the heart. CARL J. WIGGERS and A. DEAN, JR.

Exhibit of photographically recording apparatus for studying the dynamics of the circulation. CARL J. WIGGERS.

BILE PIGMENT METABOLISM

IV. Influence of Fresh Bile Feeding upon Whole Bile and Bile Pigment Secretion

C. W. HOOPER and G. H. WHIPPLE

From The George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

Received for publication October 30, 1916

This paper presents the experimental data resulting from feeding fresh bile from various animals to dogs with bile fistulae. Bile feeding causes a very definite increase in bile flow from the fistula, as is well known, but also, with few exceptions, it causes a definite *diminution* of the outflow of bile pigments in spite of active cholagogue action, which might be expected to sweep an excess of bile pigments out of the liver. This sweeping out of an excess of bile pigments during cholagogue action is exactly what we might expect, provided the liver had simply an eliminative function in disposing of worn out corpuscles and hemoglobin by way of the bile. In the following paper the various constituents of bile are tested on bile fistula dogs, and the striking effects are analysed.

That bile feeding has a definite and striking effect on the bile pigment secretion is established, and is of considerable importance in understanding this obscure story of pigment metabolism. Can we assume that bile feeding causes a decrease in the continuous disintegration of red cells which we are told is going on with such rapidity in the body, and is solely responsible for the outpouring of bile pigment? There may be a reasonable doubt on this point. It is to be recalled that most of the evidence concerning the short life cycle of the red cell is based on end product determinations. If there is a suspicion that other substances than hemoglobin may be formed into these same end products or that some of the used up hemoglobin may not be formed into this same end product, we may also question the life cycle of the red cell as determined in this manner. At least we may say that there is a possibility of error, and assume that the life of a red cell *may* be

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